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À L'OCCASION DE SON SOIXANTE-CINQUIÈME ANNIVERSAIRE
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THE MECHANISMS OF THE TRAPPING EFFECT OF ARTIFICIAL LIGHT SOURCES UPON ANIMALS

by

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(Laboratory of Comparative Physiology, University of Utrecht)

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[1] The observations which of necessity had to be carried out at sea were arranged in the bay of Naples (Italy) and the bay of Banyuls (France), partly with the aid of the local light-fishery.
The greater part of the laboratory experiments were carried out at the Laboratory of Comparative Physiology of the University of Utrecht.

I. INTRODUCTION

> "The fascination of fire by night is potent to the destruction of fish, bird, or beast..."
> W. HOUGH

Considering the immense amount of work done on photo orientation it is surprising to discover that a well known and universally utilized phenomenon as the "magnetic" power of attraction excercised by artificial light sources upon widely different species of animals like insects, fishes and birds, is still wrapped in a romantic veil of secrecy when it comes to account for the underlying physiological mechanisms.

In fact, at first sight, one might be struck by the seeming paradox that it is just nocturnal animals that completely ignore their otherwise apparent photophobia every time they exhibit this "positive phototactic" concentration around an artificial light source. It is obvious, however, that the animal's activity during the absence of daylight is a *sine qua non* for this reaction to occur, and consequently nocturnal animals are the predominant victims. But when diurnal animals, which are normally active only in the day-time, are active at night for one reason or another (migratory birds or artificially activated insects like bees) then these animals too are subjected to the trapping effect of artificial light sources. It is clear, therefore, that the peculiar point is *not* the concentration of *nocturnal* animals around artificial light sources, but the very concentration of animals *whether nocturnal or diurnal* around artificial light sources. As a corollary the basic problem is reduced to the question: what is the difference between the normal environmental illumination by day or by night—brought about by the sun or the moon respectively—and the man-made environmental illumination around an artificial source of light? In other words, why is the sun or the moon ignored by animals in so far as they do not run, swim or fly towards these light sources, whereas they do towards lamps?

The author was fascinated by this problem during the nightly collection of sardines and anchovies for the aquarium of the Zoological Station at Naples with the help of the local light-fishery technique (VERHEIJEN, 1956). It was considered that the collection of insects with lamps, and the notorious attracting power of light houses and light vessels upon birds migrating by night might constitute related or even identical problems.

The importance of the study of these problems is twofold:

a. From a theoretical point of view it may contribute to a better understanding of the mechanism of photo orientation, while,

b. from a practical point of view it may provide answers to questions inherent in the application of artificial light for the capture of insects

and fishes or in the protection of migrating birds against light houses. Because of lack of insight into the trapping effect current techniques in this field are largely based on tradition and intuition, both factors of a rather conservative character.

In the following the general features of the optimal conditions for trapping widely different species of animals with light are traced, and these features will be confronted with the results of laboratory experiments on photo orientation. The emerging working hypothesis is tested and confirmed experimentally and after that the light trap phenomenon is considered with regard to recently developed insights into the interaction between the central nervous system and the sense organs during the process of orientation in space (re-afference; feed-back in servomechanisms). The limitations that are put to the practice of the light trap technique by the nature of the underlying reactions will be put forward. Lastly attention will be focussed on the recent tendency among cyberneticians to imitate reactions like "phototropism" with the aid of electronic "animals".

I am greatly indebted to the Royal Netherlands Academy of Sciences for making available to me the Netherlands work table at the Stazione Zoologica, Naples, and to the Netherlands Government for its financial support.

It is a pleasure to acknowledge my indebtedness to Prof. R. Dohrn and to Dr. P. Dohrn for their hospitality, for putting one of the motor boats of the Stazione Zoologica at my disposal, and for their readiness in contacting the professional fishermen. I wish to express my gratitude to Prof. G. Petit, Director of the Laboratoire Arago, Banyuls-sur-Mer, for similar reasons. I also wish to thank Prof. S. Dijkgraaf for kindly reading the manuscript of this paper.

II. SOME GENERAL CONSIDERATIONS ABOUT LIGHT FIELDS AND PHOTO ORIENTATION

At an early stage in the exploitation of artificial light sources in experiments on photo orientation—during the second half of the nineteenth century—it became usual to record the responses of organisms in a darkened room while the walls and the experimental apparatus were painted dead black as far as possible to reduce "interfering reflexion". In other experiments organisms were subjected to the light field produced by the sun or by diffuse daylight behind a window. The question at issue—whether it is the *intensity* of the light stimulus alone that governs the response to light or whether the *direction* of the rays of light is the important factor—dates from the time of these earliest investigations on photo orientation of motile organisms, whether plants or animals. The

many experiments on the intricate problem of the directing action of light upon organisms — especially when the light is emanating from an artificial source — have, for more than a century, left unsolved the question of the relative importance of *direction of the rays of light* as opposed to *change of intensity*. To state it briefly and schematically the crowding of animals around a light source may have formed *a*. because the animals arrive nearest the light source after having moved along the path of incidence of the light or *b*. because the animals arrive in the brightest part of the environment after having moved up to the intensity gradient around the light source.

The former possibility is related to terms like heliotropism, phototropism and phototaxis, the latter to terms like photopathy and intensity preferendum.

Although it has been pointed out more than once that the distinction between the two reaction types is artifical and unwarranted in many respects, most workers in this field have focussed attention principally to one of the two aspects and consequently their experiments were designed more or less unconsciously in justification of their preference.

With the general confusion as to the relations between these factors in mind, there seemed to be good reasons to give some exposition of the illumination conditions in a number of light fields.

In the usual experimental circumstances the two illumination factors *direction* and *intensity* are definitely related to each other. This can easily be seen in the clear case of an artificial source of light emanating a divergent beam towards a non reflecting black environment. In a definite point in this light field the rays of light are coming from a definite direction viz. along the lines which connect the point under consideration with the light source.

The illumination intensity in this point as measured in this direction has a definite value determined by the power of the light source and the distance between the light source and the point under consideration. When measured in other directions the illumination intensity in the same point decreases according to the cosine law and reaches zero when measured in some direction perpendicular to the line which connects the point under consideration and the light source. The illumination condition in the point in question is characterized by the angular light distribution (see section v).

When the illumination conditions are considered with reference not to a point but to the surface of a globe in this light field, then the most intensely illuminated part is directed towards the light source while the illumination of this part is the more intense the smaller the distance between the globe and the light source. Consequently the illumination intensity of various parts of the surface of the globe depends on the

direction of the rays in two ways. *a.* The parts turned towards the direction from which the rays of light are coming, viz. in the direction of the light source, are the most intensely illuminated, while the other parts are in the shadow, and *b.* the illumination intensity of the first mentioned parts of the globe increases, the more the globe moves into the direction from which the rays are coming, viz. towards the light source.

The consideration of these illumination conditions on a globe is important as organisms have a more or less opaque body with a solid form. Their surface is not homogeneously photoreceptive, this faculty being concentrated substantially in some specialized regions, the eyes. It follows from this description that, in general, the illumination intensity of the eyes is unequal. The illumination intensities of two eyes are identical only in some special positions of the animal with reference to the illuminating light field. Moreover, the directive character of light results in an unequal intensity of illumination of different parts of the photoreceptive surface of any eye that has some properties of a *camera obscura*. But these important complications come up for discussion later on (see section V).

The normal relation between the direction of the rays and the change in illumination intensity as pictured above under *b.* is generally referred to as *light gradient* though this term is often used whereas the illumination conditions leading to the gradient are not clearly in mind (cf. p. 7).

In this connection attention may be drawn to the endeavour of some authors to eliminate the directional character from a light gradient as far as possible (VIAUD 1951b, BAUERS 1953a, WOODHEAD 1956, THIEMANN 1957). From their attempts it was concluded that these workers are of opinion that direction is only an interfering, and not strictly necessary, consequence of some experimental light gradient. It may be emphasized here that the term "*gradient*" implies a more or less marked directional character of the light field in question. This can clearly be seen in natural light gradients. If we confine ourselves first to natural "terrestrial" light gradients, these are found in places where direct beams of the sun play no part or at least no predominant part in the general illumination, e.g. the outskirts of a wood, inside a cave, under a tree or a stone, etc. There the sources of light are some parts of the sky and the reflecting surfaces of objects all round. The light of the sky enters the trees or the cave through a diaphragm-like opening and consequently the illumination intensity diminishes with the distance from the diaphragm. The same holds for the light reflected from objects. Here, too, the illumination intensity caused by the light from these "near light sources" decreases with increasing distance. The directional character of the illumination conditions in these gradients is easily illustrated when we consider the distribution of the illumination intensity over the surface of an opaque object placed somewhere in such a gradient under a tree or in a cave. We find that one side of the object is the most intensely illuminated while the other sides are more or less in the shadow. The same holds for the natural vertical "aquatic" gradient under water where absorption and scattering play an additional or even predominant role in bringing about the gradient.

All this is to show that "light gradient" implies a more or less conspicuous directional character of the light field. It should not, however, be overlooked that the

reverse is not true: the directional character of a light field is not necessarily indicative of a light gradient as will be evident from the light field in a parallel beam.

It is not difficult, however, to devise conditions in which the normal relations between the factors intensity of illumination and direction of the rays of light as pictured above are disturbed: both factors (in a purely diffuse light field) or only one (namely intensity in a parallel beam) can be eliminated, or one factor may be set against the other in some way (see below).

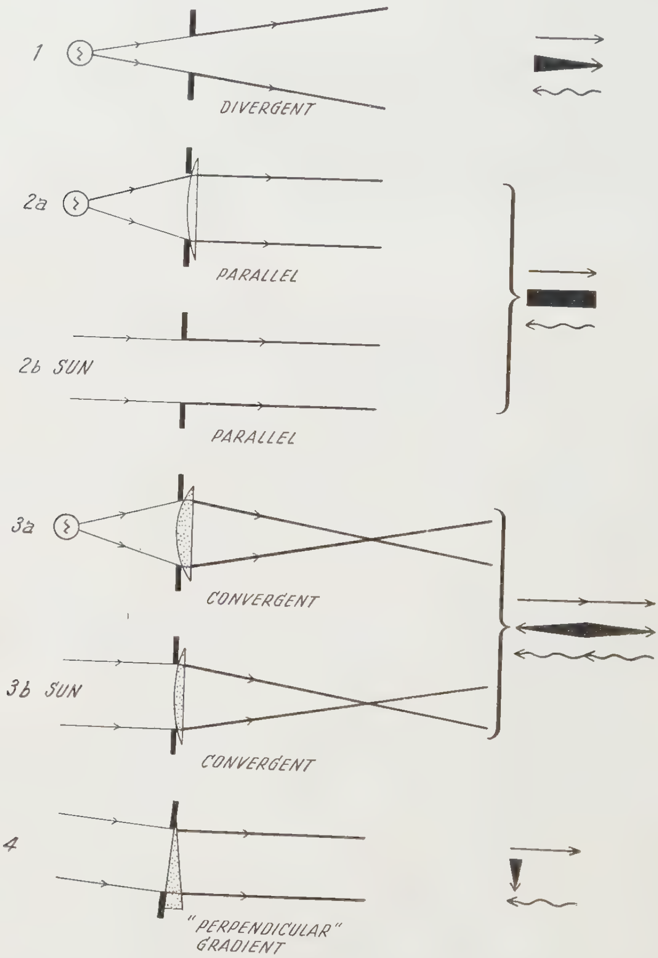


Fig. 1. The direction of locomotion of widely separated species of organisms in a number of light fields which has been used in earlier work. → direction light rays; ▶→ direction diminishing intensity; ~→ direction locomotion organisms.

The most important of the various light fields that have been used in previous work may be summarized as follows (see fig. 1).

1. *Divergent beam.* This is obtained from an artificial light source (candle-flame, electrical lamp) in a dark room with black walls, floor and ceiling. In this case the illumination intensity varies inversely as the square of the distance to the light source. Other grades of divergence can be produced by placing a convergent or a divergent lens in the divergent beam from an artificial light source or a divergent lens in the parallel beam from the sun.

2. *Parallel beam.* This is produced by means of a convergent lens and an artificial light source placed in the focus of the lens. In some experiments a beam of parallel rays of the sun was used.

3. *Convergent beam.* This is procured by using a convergent lens and an artificial light source placed somewhere outside the focal distance of the lens, the amount of convergence being determined by the distance between light source and lens. Compared with 1. an "inverted" gradient could be seen in this light field.

4. "*Perpendicular*" *light gradient.* With the aim to obtain an intensity gradient at right angles to the direction of the rays of light, some workers have employed a triangular glass dish filled with an absorbing substance like diluted Indian ink, or some kind of screen of graded absorbing capacity, generally referred to as "gradient-apparatus".

5. *Illumination conditions that escape closer description.* In many experiments the illumination conditions are some mixture of several light fields. Some efforts to collect insects or fishes with the help of light sources fall under this group. But the same holds for the light fields used in many laboratory experiments (e.g. "light gradients").

Although the light fields No. 1 and 3 no doubt meet the features of a gradient as pictured above (p. 5), most authors do not refer to it as a gradient. This may be partly due to the fact that animals placed in such a light field frequently do not show the characteristic features—search for their intensity preferendum—of photo orientation in a gradient, as will be seen later on.

III. PREVIOUS WORK

The response of animals to light stimuli has been the subject of much study on the part of early physiologists and psychologists. During the second part of the nineteenth century there was a gradual increasing interest in the reactions of motile organisms in response to light stimuli, first by botanists and later on by zoologists. Since then, the stream of papers has continued incessantly, but to date, unfortunately, no one has succeeded in gathering the fundamental facts necessary for an under-

standing of the physiological mechanisms underlying the trapping effect of artificial light. It is of interest, therefore, to note the historical development of the concepts of photo orientation. This may prevent us from making mistakes similar to those made by others in ignorance of, far from recent, but nevertheless relevant data. A great number of data are derived, for this purpose, from four main fields, namely *A* laboratory experiments on photo orientation, *B* the fishery with light sources, *C* the protection of migrating birds against light houses, and *D* the collection of insects with light traps. We are confronted here with one "purely scientific" and three "applied" fields. Their respective students have led a rather independent existence. In this way it is understandable that in a recent and extensive review on fishery with light sources (SCHÄRFE, 1953) not one significant reference to one of the other fields can be found. Similarly, one might conclude from papers on light traps for collecting insects, that insects are the only animals that are subjected to this trapping effect. Superfluous work, inappropriate methods, and unjustified conclusions are the inevitable results of this situation.

A. LABORATORY EXPERIMENTS

Many species of organisms placed in the above light fields frequently show the peculiar tendency to move into the direction from which the rays of light are coming, irrespective of other aspects of the light field, such as intensity. Several workers have tried along widely divergent lines of thought to incorporate this tendency—as far as it is recognized at least—in their theories on photo orientation.

No doubt, the most "universal" and at the same time most vague and primitive theory was the opinion that the orientation phenomenon is in some way controlled by the direction in which the rays of light energy pass through the organism's tissues. This theory was advocated by SACHS for plants, and, at least in the beginning, by LOEB (1888) for animals as well. Fundamentally different was the opinion of DE CANDOLLE and that of VERWORN (1895), both maintaining that the different intensity of illumination on different parts of the photoreceptive surface of the organism would be the determining factor in plants and animals respectively. Later on LOEB abandoned his original view in favour of this intensity theory[1]. While used by many workers on photo orientation, the concept of intensity drifted from the distribution of illumination intensities on photoreceptive surfaces into the wider and more ecologically tinted sense of the distribution of intensities in the space around the organism in question. According to the original

[1] For the earliest development of the theories on photo orientation see for instance BUDER 1917, MAST 1938, FRAENKEL and GUNN 1940.

theory the organism moves into some particular direction because of the momentary distribution of light intensities on its photoreceptive surfaces, while, according to the other view, the organism moves towards a place in the environment where the movement stops as soon as a certain light intensity is reached. This place is called the "indifferent zone" and the intensity there the "optimum intensity" or the "intensity preferendum".

It will be clear from what has previously been said about the illumination conditions at the surfaces of an object in a natural light gradient, that in both theories the direction of the animal's movement would not necessarily be different, as the intensity distribution on the animal's photosensitive surfaces results unambiguously from the intensity distribution in the animal's environment. But it should be borne in mind that the distribution of intensities on the animal's photosensitive surfaces is the only direction indicator available to the animal, while the ultimate result of the animal's movements with regard to the light intensity to be reached in the environment—however much it may be in accordance with the direction indicator—is nevertheless best of all characterized with the expression "wait and see".

The fact that these two fundamentally different aspects of photo orientation—the momentary orientation and the ultimate result of the orientation—has not always been clearly discriminated, frequently led to great confusion of ideas, as is demonstrated further on.

The photosensitive surfaces of organisms are widely divergently developed from very primitive eye spots with weakly developed directional sensitivity up to the vertebrate eye in which this faculty is highly developed. The animal's movements are, accordingly, less or more precisely controlled. The physical basis of the control of these movements is the rectilinear travelling of light.

Although equipped with primitive eye spots many flagellate protozoans show conspicuously directed locomotion in certain light fields. From his classical experiments with the swarm-spores of several algae in a gradient-apparatus (see p. 7), STRASSBURGER (1878) arrived at the conclusion that these organisms move in a direction parallel to the incident light rays and that they cannot move in any other direction. He introduced the term *phototaxis* to indicate these movements. But from his experiments in convergent and divergent beams of light he further distinguished between *a. photometric* organisms that are *photophyl* or *photophob* depending upon the light intensities in a normal gradient (fig.1, 1) and other factors (for instance the stage of development) and *b. aphotometric* organisms that move towards the light source even when the intensity is diminishing, or from the light source, even when the intensity is increasing in this direction, as is the case in the "reversed" gradient

of a convergent beam (fig. 1, 3a, b). Other authors termed the movements which are influenced by the total of light intensities en route "*photopathy*" (GRABER 1883, and others). This anthropomorphically tinted term is still in use (VIAUD 1949, 1951a, 1951b, 1956).

Working with *Volvox* and other motile plant forms in some kind of perpendicular gradient field (fig. 1, 4), OLTMANNS (1892) concluded that these organisms are attuned to a certain intensity of light and that their movements, whether parallel to the rays or not, are directed towards their intensity optimum.

With STRASSBURGER and OLTMANNS we are confronted with the classical controversy which might be termed the direction–intensity controversy. It will be evident from what has previously been remarked that this is rather confusing since the direction, in turn, is gathered by the organism from intensities. Numerous experiments have been performed in support of either of these views, but only some of them will be mentioned here. The number of workers attempting to demonstrate the paramount importance of "intensity" is relatively small. YERKES (1899) studied the behaviour of small Crustacea (Branchiopoda, Ostracoda) and GOLDSMITH (1921) the movements of marine planktonic animals like *Mysis*, by using some kind of gradient-apparatus. From the movements of the experimental animals towards the brightest region they both concluded that the reactions to light are not the result of an orientation to the direction of the rays, but that the organisms are moving towards a preferred light intensity.

Similar experiments with a gradient-apparatus, and with divergent and convergent beams were carried out by DAVENPORT and CANNON (1897) with *Daphnia*, TOWLE (1900), HOLT and LEE (1901) with Protozoa, MOORE (1909) with Nauplii of *Balanus* (Cirripedia), BUDER (1917) with a great number of Flagellata, CLARKE (1930) with *Daphnia*, KALMUS (1931) with larvae of *Sabellaria* (Polychaeta) and SPOONER (1933–34) with marine plankton organisms including fish larvae. They all criticized the intensity theory in one way or another. TOWLE (1900) stated that when the sunlight falls through an Indian ink prism, light conditions in a trough behind the prism are not so simple as they seem to be. An organism placed at the dark end may be influenced by 1. the rays which pass through the thick end of the prism, and 2. the diffuse light scattered and reflected from the brightest region. The relative value of these two components will therefore determine their resultant effect, and hence the direction of the response of the organism. To an individual organism placed in the darker part of the trough the source of light is the lighter end of the trough, and towards this it moves. The source of light is only another name for the point or region from which the most intense rays come. The animal is only moving in the path of

the most intense rays that strike it, and is then still *phototactic*, not *photopathic*. HOLT and LEE (1901) argued that the intensity of the light determines the sign of the response (positive or negative) while the part of the body stimulated—determined by the direction of the light decides the ultimate orientation of the organism. MOORE (1909) stated that the Nauplii of *Balanus* move towards the light at low intensities, and away from high intensities. He described the movements of his experimental animals in convergent and divergent beams somewhat like this: the positive organisms swam from the most distal part of the light field towards the caustic in divergent light, i.e. in light of increasing intensity, and then onward in converging light, i.e. in light of decreasing intensity. His conclusion from these reactions is clear enough to be worth quoting. "At first sight it looks proven from this that the intensity of light is of no effect, and the direction of incidence the whole matter, because the organisms appear to swim in one direction indifferently, whether the illumination is increasing or decreasing. In reality, however, such a conclusion would be fallacious, for in order that, say, a positive organism should turn when it began to swim in light of gradually decreasing intensity, it would be necessary for it to turn its sentient surface away from the light, and that would plunge it into darkness." Finally he tried to view related subjects under this aspect: "...the whole matter depends upon the force of the stimulus outweighing the degree of development... of the nervous system, or, in more general terms still, the co-ordination of the organism". He continued: "It is such excess of stimulus over organization which makes the moth burn itself in the flame or the bird dash itself to pieces against the light house lantern..."

At first sight this reasoning might seem justified, but on closer examination it will be clear that it is a fallacy. For the moth and the bird are flying onward in divergent light towards a lamp. They are flying, consequently, into the direction from which the rays are coming in light of ever sharper rising intensity, a situation which is not comparable with the convergent beam. The movements of organisms in a convergent beam might be attributed to the impossibility to reach a certain intensity necessary to stop the directed movement. This is the result of the inversion of the intensity gradient with respect to the direction of the rays in the convergent beam. In the divergent light around a lamp the situation is exactly reversed. It is incomprehensible why the moth or the bird has to move towards the light source without high light intensities or other undoubtedly injurious stimuli being able to hold back the animals from a further approach to the light source.

From an unprejudiced observer of the animal's movements under these conditions the notorious words "forced movements" escape involuntary. These two words are virtually identified with the name LOEB

and with the protracted and fruitless discussions in which these words were used in contradistinction with the words "free will". To recent investigators in this field the words "forced movements" are only of historical value (however see VIAUD), and every attempt to revive the term even seems to have been considered offensive (GUNN 1950). However, sound intuition described this type of reaction quite rightly, as will be seen. The term "forced movement" must, however, not be used as opposed to "free will". Its re-introduction will be justified theoretically and experimentally in contradistinction to "normal orientating behaviour".

Some experiments with totally different animals, the results of which run parallel in a remarkable way in this connection, are pictured below.

First an experiment of YERKES (1900). A trough with water was set in front of a window with its long axis perpendicular to the window. Into the end towards the window ten drops of strong HCl were put; into the opposite end twenty *Daphnia*. The animals all moved towards the window and swam directly into the acid. On first reaching the acid zone they zigzagged about a little and increased their activity, but none turned back. In a few seconds all were dead. When the trough was turned parallel to the window there was a marked difference in reaction. Occupying the available space the animals spread themselves as far as the edge of the acid, and while occasionally one entered it, the majority turned back and avoided the stimulating substance. YERKES concluded that it is evident that the directive influence of light is surprisingly strong, being sufficient to lead an animal to its destruction.

VON BUTTEL-REEPEN (1900) placed a bee in an open test-tube. The test-tube was laid down behind a window so that the longitudinal axis of the tube was perpendicular to the plane of the window and the closed end was directed outside. The bee died after having tried for hours to escape through the closed window side of the test-tube.

HOLMES (1901) studied the phototactic reactions of terrestrial amphipods like *Talorchestia longicornis*. During the day the animals lie buried in the sand, but at night they come out to feed. If then a lantern is placed in their midst they are attracted to it in large numbers. When walking about over the seaweed individuals may be seen to stop as soon as they are affected by the light. They quickly turn and face the lantern and then they make straight towards it, either by walking or by a series of leaps. When *Talorchestias* in a glass dish are exposed near a window to direct sunlight or to direct sunlight reinforced by light reflected by a mirror they walk or hop to the "positive end" of the dish and struggle there until overcome by the heat of the sun. The animals do not go to the "positive end" of the dish in order to get from a darker into a lighter area; they will go to this end just as well when it is made somewhat darker than the other by coloured glass.

In an attempt to arrive at some interpretation of these reactions HOLMES argues: "The behaviour of *Talorchestias* naturally produces the impression that they prefer to rest quietly in the shade but are drawn out by the phototactic impulse apparently against their will and set going in the direction of the rays of light". About the same results were obtained in experiments with *Orchestia agilis*. But to make things still more confusing these animals became strongly negative phototactic when brought into a dimly lighted room! Evidently HOLMES did not know how to interpret the paradox of positive phototaxis towards strong light and negative phototaxis in dim light.

No doubt the most notorious of this type of experiments are those made by LOEB with the small gregarious caterpillars of *Porthesia chrysorrhoea* (LOEB 1890, 1905). Judging from the many times it has been criticized in the literature, the following experiment seems to have been highly offensive. About a hundred specimens of the caterpillar were put into a test-tube. The test-tube was placed on a table such that the longitudinal axis of the tube was perpendicular to the plane of the window and in such a way that the open half was in direct sunlight, while the closed half that was nearer to the plane of the window was in "diffuse" daylight. The animals passed from the direct sunlight into diffuse daylight without even attempting to return into the sunlight. According to LOEB the orientation of the animals was determined by the direction of the rays of light, and not by differences in the intensity of the light in different parts of space. The caterpillars died from starvation when there was food only at the open end of the tube further from the plane of the window.

LOEB's experiments with these caterpillars were repeated by HESS (1919), DEEGENER (1921), MANQUAT (1921a, 1921b), SCHMITT-AURACHER (1923), LAMMERT (1926) and others, while GOLDSMITH (1921) made similar experiments with marine planktonic organisms. With the exception of HESS and SCHMITT-AURACHER none of them could reproduce the results of LOEB. HESS supposed that under natural circumstances many caterpillars would die from starvation at the end of leafless twigs just as in his experiments and in those of LOEB. DEEGENER (1921) and MANQUAT (1921a, 1921b) experimented with the caterpillars not only under laboratory conditions but in their natural habitat in trees as well. They always found that caterpillars on leafless twigs turned in search of twigs with leaves irrespective of light conditions. DEEGENER emphasized, however, that the results of LOEB and HESS should not be attributed to phantasy, for the reactions of the caterpillars could have been the product of extreme laboratory conditions.

As usual the character of these laboratory conditions remained in the dark. The neglect of DEEGENER's warning is an essential shortcoming of

many of LOEB's opponents. On the other hand, DEEGENER pointed to the fundamental fallacy made by LOEB when he extrapolated from the reactions of animals under laboratory conditions to their normal behaviour under natural circumstances.

The explanation of the contradictory results of the above mentioned authors is not obvious. On repeating the experiments of LOEB they state that the experimental conditions were exactly the same but in most cases it is not possible to ascertain the correctness of this assertion from the papers reviewed above. As is seen later on, the characteristic "forced movements" are the result of peculiar illumination conditions which are characterized by the absence of scattered and reflected light (see section V A). Now, in certain experiments, the light conditions were obviously different from LOEB's. GOLDSMITH (1921), for instance, used white walls at the room side of her experimental tanks in order to make light conditions more diffuse, as she argued. But these light conditions are exactly opposite to those touched on above. The majority of the experiments of other authors are open to criticism in similar respects. The mere statement that experiments were performed behind a window gives hardly any cue as to the actual illumination conditions. In studying the reactions of animals in light fields it has often been neglected that conclusive results can only be expected from experiments under delicately controlled illumination conditions. This is the more cogent when it is not clear which aspects of the environmental illumination are of importance in bringing about the reactions of the experimental animals.

Unaware of these aspects of the problem THOMAS (1954) devoted some theoretical considerations to the reactions of the caterpillars in LOEB's test-tube by comparing these reactions with the behaviour of the caterpillars in their natural habitat, and he reverted to similar considerations of MANQUAT (1921 b). According to THOMAS the results of LOEB's experiments are due to far-fetched features of the experimental conditions like the fact that the very narrow tube was placed exactly in the desired direction. Moreover, THOMAS argued, that the smoothness of the curved inner surface of the tube and the overheating of a part of the tube by the sun would have been important factors. This last argument was also put forward by ERHARD (1923). According to CHAUVIN (1956) the behaviour of the caterpillars in LOEB's experiments might be compared with that of an Eskimo transported to the Sahara, a comparison which is anything but elucidatory. The explanation of LOEB's experiments given by GRISON (1957) is not satisfactory either.

THOMAS and others claim that LOEB devised his experiments in order to justify his forced movement theory, while, in addition, he did not point to other results. But it can be argued against these opponents of LOEB that the crucial point is in fact that it is possible in one way or an-

other to devise experiments in support of the forced movement idea, as we have seen above (HESS, SCHMITT-AURACHER, and the many experiments in "abnormal" light fields mentioned earlier). After perusal of LOEB's relevant papers (1888, 1890, 1905, 1918) we can only conclude that the peculiar results of his experiments can hardly be attributed to accidental factors as suggested by several opponents—his various control experiments preclude such easy attacks on error—but that they must be due to more deeply seated causes.

In this connection it seems justified to expound the forced movement theory or tropism theory of LOEB, and together with it the abundant criticism it elicited. For this purpose two different aspects of this theory will be distinguished, namely *a*. LOEB's description of the movements of animals as observed in experiments under certain illumination conditions, and *b*. his attempt to give a universal theory to account for these and other movements of animals in answer to light and several other stimuli.

A general impression of his ideas can be derived from his following words (LOEB 1918): "Motions caused by light or other agencies appear to the layman as expressions of will and purpose on the part of the animal whereas in reality the animal is forced to go wherever carried by its legs. For the conduct of animals consists of forced movements. The term forced movements is borrowed from brain physiology, where it designated the fact that certain animals are no longer able to move in a straight line when certain parts of the brain are injured, but are compelled to deviate constantly towards one side... If we bring about a permanent difference in illumination of the eyes, e.g., by blackening one eye in certain insects, we can also bring about permanent circus movements. This shows that animal conduct may be justly designated as consisting of forced movements".

LOEB pointed to the bilaterally symmetrical structure of many animals. In his opinion their body is divided into a right and a left half which were thought to be fairly independent. The morphological plane of symmetry of an animal was considered to be also its plane of symmetry in physiological or dynamical respect. The stimulus intensities on symmetrical elements of the surface of the body, e.g., the sense organs, induce certain processes in both halves of the nervous system, which in turn regulate the tension of symmetrical muscles in the locomotor apparatus and through that the direction of movement of the animal.

As for the movements of an animal towards a light source LOEB will be quoted from his last paper on heliotropism (LOEB and NORTHROP, 1923) in which he mainly refers to his first paper on this subject (LOEB 1888). We read: "...if there is only one source of light, certain animals are automatically orientated by the light in such a way that their heads are turned towards the source of light and that their planes of symmetry are turned into the direction of the rays of light. In that case, the animal is automatically compelled to move to the source of light". LOEB continues: "If there is only one source of light, and the plane of symmetry of the animal goes through the source of light, the symmetrical eyes (or symmetrical photosensitive elements of the skin) are struck by the light at the same angle, and the intensity of illumination is the same for symmetrical elements. When, however, symmetrical elements of the eyes (or skin) of the animal are no longer struck at the same angle by the source of light, e.g. when the animal is illuminated sidewise, the intensity of illumination by the

source of light is no longer the same for the symmetrical retinae or other symmetrical photosensitive elements, and the animal is automatically compelled to change the direction of its motion in such a way that its plane of symmetry is again brought into the direction of the rays of the light. As soon as this happens, the animal will be compelled again to move in a straight line towards the source of light".

The only words with a somewhat interpreting tendency in these sentences are the words "automatically" and "compelled", for the rest these sentences are a fairly accurate description of experiments of the kind mentioned before.

But we are not left in the dark as to how the words "automatically" and "compelled" are to be taken in LOEB's "forced movement" conception: "As long as the rate of photochemical change in symmetrical parts of the photosensitive organs is the same, the tension of symmetrical muscles in the locomotor organs—legs, wings, or swimmerets—will be effected in the same way and the animal will continue to move in as straight a line as the imperfections of its locomotor apparatus permit. When, however, the rate of photochemical change is no longer the same in symmetrical elements of the eye or the skin, the tension of the symmetrical muscles of the locomotor apparatus will no longer be the same and the direction of the motion of the animal will be automatically changed... What appeared to the earlier investigators as a mysterious attraction of the animal by the light... thus turned out to have been only a case of automatic orientation of the animal due to a photochemical effect on the retina or other photosensitive elements of the surface of the animal".

As is evident from LOEB's words the animal is steered by *quantitative* differences in movement of symmetrical organs of the locomotion apparatus. This is caused by *quantitative* differences in stimulation intensity of symmetrical photoreceptors in a way which was obviously supposed to be based upon a single junction-function of the nervous system between receptors and the muscles of the locomotor organs. It seemed to LOEB that the actual construction of an artificial heliotropic machine by HAMMOND and MIESSNER (see LOEB 1918) not only supported his mechanistic conception of the volitional and instinctive actions of animals in general, but that it also proved his theory of heliotropism, since his theory served as basis in the construction of the machine. Strictly speaking LOEB's last conclusion was not quite true, because steering was not done by quantitative differences between the rotation rate of the right and the left locomotor wheel—as is sometimes pretended in the literature—but by an additional steering wheel, though this does not materially alter the performances of the machine. The machine would follow a light all around the room in many complex manoeuvres.

It may be assumed to be generally known that LOEB's conception has been rejected as being far too schematic for an all-round elucidation of animal behaviour in general and that it is even incapable of giving an explanation of seemingly simple movements of animals, as for instance, the walking of insects towards a light source. The numerous theoretical

considerations contra LOEB will be passed over for the greater part (see for instance JENNINGS 1910, BALDUS 1927). Attention will be given mainly to some papers pointing to obvious shortcomings in his theory, and especially to those which are pertinent to our problem. Asking "What are tropisms?" MAST (1914) could already sum up some twenty different senses in which the term "tropism" had been defined up to then. He pointed to the fact that the term "tropism" had also been endowed with mystical causal powers. As will be seen (p. 28,29) this mystification is still met with nowadays. MAST advised to substitute terms of a more precise meaning. He suggested the term "negative" or "positive reaction to light".

Such a term is purely descriptive and gives no indication as to why and how the movements are performed. Recently this term has been used by BAUERS (1953 a) and THIEMANN (1957) to indicate the movements of organisms towards a light preferendum.

According to VON BUDDENBROCK (1915, 1916) the lateral orientation of crabs walking sidewise to a light source is incompatible with LOEB's schematical tropism concept.

Earlier HOLMES (1908) argued in a similar way but he found in addition that, when the fiddler crab (*Uca pugnax* Smith) had reached the light source the animal usually did not maintain its lateral orientation but that it faced the light. Moreover, he made experiments in which the long eye-stalks of the crab were crossed like the parts of the letter X and tied in the middle. Crabs treated in this way showed great confusion in their reactions to visual stimuli. When approached by something they often ran towards it instead of away from it as they usually do from all moving objects. Often they moved haphazardly as if the result of their movements were something unexpected. The animals often sulked as if discouraged with their efforts. He concluded that "we are not in a position to give a satisfactory explanation of orientation to the direction of the rays of light". These experiments bear a striking resemblance to those of MITTELSTAEDT (1949) leading to the formulation of the re-afference principle (VON HOLST and MITTELSTAEDT 1950).

VON BUDDENBROCK (1915, 1916) pointed quite rightly to the fact that an explanation of the turning of a bilaterally symmetrical animal around its transverse axis—necessary for instance to reach a light source above or below the plane through the longitudinal and the transverse axis of the animal—is impossible in terms of LOEB's theory.

LOEB was acquainted with such movements or movement intentions through the experiments with insects of AXENFELD (1899), HOLMES (1905) and GARREY (1917, 1919). After blackening of the lower halves or the upper halves of both eyes of the robber fly *(Proctacanthus philadelphicus)* GARREY found that the insect showed peculiar positions with the head tilted upwards or head bent down respectively. When trying to fly, "looping the loops" were performed backwards or forwards respectively. But LOEB's interpretation of these movements was unlike that given of the widely reported circus movements of animals with one eye covered. While these circus movements

were put forward in evidence of the forced character of turning movements around a vertical axis, the results of the experiments of GARREY and others were used to show that there are two more groups of forced movements, namely, forward and backward movements. The whole forced movements system was pictured as follows (LOEB 1918): "The experiments on the effect of blackening different parts of one eye indicate that the different parts of the retinae of positively heliotropic insects are connected in a simple way with the main centers of the three types of forced movements: namely, the left eye is connected with the brain center causing motions from right to left (and the right eye with the center for the opposite motion); the lower halves of the retinae with the forward movements, the upper halves with the backward movements". This conception never made clear how these last two types of forced movements play their role in bringing about the forced movements of animals towards a light source.

VON BUDDENBROCK (1915, 1916) was of opinion that the movements of an animal towards a light source are the result of the action of the reflex arc eyes–nervous system–locomotor organs. This is a purposeful adaptive structure of the organism necessary for the tasks of maintenance and reproduction, and making the tropism theory an entirely superfluous doctrine. But for obvious reasons VON BUDDENBROCK was not quite certain of the purposefulness of the mechanism involved in the movements towards a light source. For in this case the tropism seems useless or even harmful to the animal. He attributed this to the unnatural conditions during the experiments in question. Moreover, he suggested that it might be imagined that in animals with higher developed eyes the forming of images alone is the purpose of the eyes, while heliotropism should be a by-product unintended by nature. With this suggestion VON BUDDENBROCK was very close to an elucidation of the trapping effect of artificial light (cf. section VB) but probably he was unconscious of it as appears from the fact that he searched in a different direction in 1917 (see p. 24).

Extensive and carefully controlled observations, which subscribe to the above mentioned objections of VON BUDDENBROCK (1915, 1916) against LOEB's tropism theory, were made by MAST (1923 a, 1923 b) on the drone-fly *Eristalis tenax*, and the robber-flies *Erax rufibarbis* and *Proctacanthus philadelphicus*. He demonstrated that animals with one eye covered, as well as animals with the front and the middle legs on one side removed, orient obliquely to the light, but in spite of this fact they go directly towards the source of light. In orienting on the wing, Eristalis turns up or down as well as to the right or to the left. These and other facts supported his idea that orientation is not the result of some sort of balance between the action of symmetrically located receptors and appendages on opposite sides of the body as postulated in the tropism theory.

MAST concluded that photic orientation is brought about by a series of coordinated reflexes depending upon the localization of the stimulus

in the eyes. When the insect moves towards a light source the orientating light stimulates the frontal ommatidia of both eyes. These ommatidia look straight ahead and lie parallel to the longitudinal axis of the body. In each eye there are a few ommatidia nearer the animal's longitudinal axis which look across to the other side so that the visual fields of the two eyes overlap. When stimulated these last mentioned ommatidia initiate turning to the contra-lateral side. The ommatidia of the great lateral parts of each eye initiate ipsi-lateral turning. This reflex-map of the eyes accounts for the orientation towards a light stimulus in normal animals or in animals with only one functional eye. Like VON BUDDENBROCK, MAST was of opinion that the orientating reactions are fundamentally adaptive. He admitted, however, that there are useless non-adaptive orientating reactions to light in insects, reactions which prove fatal in countless instances. As usual, this was put down to abnormal conditions, without further allusion as to the nature of the abnormality in these conditions.

The kind of eye described above is called two-way eye, because it can initiate turning in either direction. This telotactic orientation mechanism as a whole is strongly reminescent of the fixation mechanism of the vertebrate eye. Consequently the anterior region of the insect eye where the ommatidia are situated, which are not concerned in initiating turning, is often called the fixation zone and it can be compared with the fovea or area centralis of the vertebrate eye.

In comparison with the orientation in the horizontal plane, less attention has been paid to the orientation in the vertical plane. This is especially of importance in swimming or flying animals. After the work of MAST mentioned above, the mechanism of the orientation in the vertical plane has more recently been worked out by BAUERS (1953b) in bees and other insects by a technique used earlier by PRECHT (1942). When the insects were placed on a table at some distance from a lamp above this table, they walked straight on in the direction of the lamp (also with one eye covered) till the eyes were struck by the light at a certain angle. At this point the insects tried to fly from the table towards the lamp. BAUERS concluded that only the ommatidia within a certain central area of each eye are suited for the fixation process. The peripheral ommatidia of each eye initiate turning movements of the animal by which the light stimuli are brought back to the fixation region.

Fixation mechanisms in the eyes of insects were also found by DOLLEY and WIERDA (1929) in *Eristalis tenax*, by CLARK (1928, 1931, 1933) in *Notonecta glauca* and *Dineutes assimilis*, and by LÜDTKE (1935) in *Notonecta glauca*.

CLARK observed that an insect with one eye goes directly towards a light source in a dead black environment. If a white environment is

provided the insect continuously makes circus movements. In a gray environment the insect takes a diagonal path in the beam of the light source. He concluded that this diagonal path is due to a balance between the effect of the light stimuli directly from the light source stimulating the antero-median ommatidia and tending to cause the insect to turn towards the light source, and the effect of the light reflected from the background stimulating the lateral and posterior ommatidia and tending to cause the insect to turn from the light source.

In a well-screened directive horizontal beam the insect with one eye covered moves directly towards the light source because the reflection from the background produces only weak light stimuli which fall below the threshold of stimulation of the lateral and posterior ommatidia and are thus ineffective in causing circus movements.

No doubt the fixation mechanism of the eye plays a role in the telotactic walking of insects towards a lamp in a dark room. This type of phototactic movement is characteristic for what are generally referred to as "lower animals". There are, however, some instances of similar movements in vertebrates. Among vertebrates the amphibians appeared to offer particularly favourable material for the study of this kind of reactions. Moreover, they demonstrate the complicated character of these reactions.

PARKER (1903 a) found that *Rana pipiens* in a dark room with blackened walls turned and walked towards a lamp. With high light intensities, it was remarkable how persistently the frog would face the source of light and jump in that direction, "even when the light was unbearably strong to the human eye". Similar results were obtained by TORELLE (1903). She observed that, whether moving or at rest, the median plane of the body of the frog was always parallel to the incoming rays of light. In addition she found that when a frog in a box, the inner surfaces of which were painted a dull black, was lighted from above by a lamp, the response to the direction of the incoming rays was immediate: the body was raised to an angle of 45–60° to the horizontal and frequently the frog jumped upwards towards the source of light.

These results suggest that fixation mechanisms in the eyes are involved in these reactions. But PARKER (1903a) observed that eyeless frogs, like those with eyes, do move towards a light source in a dark room, though along a less straight path. Similar experiments with frogs were made by PEARSE (1910). From the fact that frogs with one eye covered moved towards the side of the uncovered eye, PEARSE concluded, in contradistinction to TORELLE, that the photic reactions are due to intensity differences in the illumination of the two eyes, and that the direction of the light is apparently of no significance. From experiments with local skin illumination PEARSE reached a similar conclusion.

Parker (1903a) observed the walking of frogs to light not only in response to artificial light stimuli, but to what he called "natural" light stimuli as well. Frogs with or without eyes behind a window turned and walked almost immediately towards the sun in low horizon.

Graber (1883) had found earlier that *Rana* sp. and *Bufo* sp. tended to come to rest in the shadow in "diffuse" light. Torelle (1903) made tests out-of-doors with frogs free to move from the sunshine into a shadow or vice versa. There was a decided movement from the sunshine into the shade. In contradistinction to their behaviour with regard to artificial light sources, the walking of the animals was not influenced by the direction from which the rays of the sun were impinging upon the animals. Each frog moved in his own direction in accordance with the animal's position with regard to a shadowed spot.

In the experiments with amphibians described above we are once more confronted with the direction–intensity controversy, and moreover, with the two aspects of intensity in orientation, namely the illumination intensity of different parts of the animal's photosensitive surface and the light intensity in different parts of the animal's environment respectively. Again, there is a gap between the reactions of the animals towards a light source whether a lamp or the sun under "artificial" illumination conditions and their behaviour in a "naturally" illuminated environment.

Strikingly similar results were obtained by Fletcher *et al.* (1916) in experiments with chicks. After hatching in the incubator the animals were tested with a lamp in a dull black environment with a minimum reflective power in a dark room. Rather more than half of some tens of animals reacted positively. Turning and facing the light source, they walked directly to the lamp and sometimes even placed their bill against the lamp. Prior to this test, an apparatus was used whereby much light from the stimulus was reflected from the walls of the experimental room. Under these conditions only a single chick walked towards the light source, while the others made random movements. These contradictory results were not further analysed as the experiments were conducted with a quite different object in view.

Similar observations may have been described by others, but most of this work has disappeared from the recent literature, obviously because such "odd" results do not fit in with current views. The author found only accidentally the few examples described above.

After reviewing the work of Loeb together with the enormous amount of work pro and con, we may now return to our starting-point to the discussion of this literature (p. 15), where we expressed the opinion that experimental results and theories must be clearly distinguished. Although it might be argued that this thesis is universally

accepted, the opposition against Loeb obviously constitutes an exception to it. Together with the rejection of his broader conception of the forced character of animal behaviour in general, and the rejection of his more limited elucidation of the movements of animals towards a light source under certain experimental conditions, the peculiar "forced" character of these movements—which are generally called "positive phototactic"—was denied or at the least overlooked. The recent theory of photic orientation developed by Viaud is an exception in that it is based on the forced character of positive phototropism (for a discussion of this theory see p. 28).

The result of the just reviewed development has been *a.* that a number of experiments mentioned before to illustrate the "forced" character of the movements of animals parallel to the direction of the light rays in different light fields, has fallen into oblivion, *b.* that a good many workers have in later years taken the trouble to study whether animals move towards lamps tropotactically or telotactically or otherwise, without having a clear idea of the importance these reactions might have in natural photic behaviour, and *c.* that when the exceptional character of the "walk towards the light" was recognized, theories were framed without taking into account the results of a number of previous experiments relevant to the problem.

The development of the ideas in this field will be reviewed and their usefulness will be tested as far as necessary.

Romanes (1883, 1886) used an anthropomorphic terminology to account for the flight of insects into a flame at night. He compared these reactions with that of birds when they dash themselves against the window of a light house, and with the concentration of fish around the light of a lantern. He supposed that the animals go in search of the light out of curiosity. They do not trouble about the sun or the moon as they are accustomed to these objects.

It was anthropomorphic considerations of this kind against which Loeb turned sharply. Rádl (1903), however, pointed out that Loeb's theory cannot explain the fact that insects do not fly towards the sun or the moon but do fly towards a lamp. As a tentative explanation he stated quite clearly that during the darkness of the night the insect *only sees the light of the candle flame* while by daylight the animal not only sees the light of the sun *but in addition the light reflected by objects in its environment.* In his view the flight of the moth into the flame is an uncoordinated action resembling the movements of an inexperienced cyclist who steers towards a cart because it is fixed obstinately before him though he desires to steer clear of it. It will be seen later on (see section v) that these considerations are to the point.

Parker (1903b) observed that the mourning-cloak butterfly *Vanessa*

antiopa creeps or flies towards an artificial source of light. He believed that the butterfly stays near the ground on bright sunny days, and does not fly towards the sun, because its flight is directed by large retinal spots rather than by small ones, even though the latter are, like that of the sun, of vastly greater intensity. In some theoretical considerations, PARKER (1922) assumed that the forced tropisms underlie the kind of complicated reaction that is regarded as spontaneous and volitional. In other words, the normal reaction of insects to light—viz. the fact that they do not fly towards the sun—is built up on a background of phototropic activity—viz. the fact that they do fly towards a lamp in a dark room. The development of eyes forming detailed images and the development of a complicated central nervous system would result in a complicated behaviour and this in turn would obscure and hide the original simple tropic scheme. The picture which emerges from these considerations bears a marked resemblance to the more recent ideas of VIAUD (see p. 28).

FRANZ (1913) was struck by the prolonged and unconditioned character—"Bedingungslosigkeit"—of the movements of a number of marine planktonic organisms towards the light. Evidently he was not aware of the fact that these reactions are, on the contrary, the result of certain conditions, namely the light conditions in laboratory experiments. From experiments with fishes he developed the idea that positive phototactic movements are evoked under laboratory conditions as flight reactions. This idea was criticized by some authors—for instance HESS (1913)—but it has been adopted by BUIJTENDIJK (1932), BIERENS DE HAAN (1940), and others.

Anticipating the fishing experiments with lamps (see section IV B), it may be pointed out here that fish concentrating around a lamp often disappear for some time when frightened by noise, predators etc. In other words their flight is directed away from the lamp.

When animals move towards a light source, then, according to HESS (1919) the animal must attain higher light intensities, just as the human being does when walking towards a window in order to read better. But, if animals would fly towards the sun or the moon, the perceived light intensity would not increase and therefore they do not fly towards these heavenly bodies.

In this way the problem is reduced to a matter of gradient. It will be clear that HESS did not reckon with the earlier experiments with "inverted" and "perpendicular" light gradients. It is evident from his further considerations that he did not know how to interpret the results of such experiments.

According to DEMOLL (1917) the flying of insects towards a light source is not caused by the light source in itself but *by the darkness of the*

environment. When the eyes are light adapted by the strong stimuli of the light source, then the insects can no longer discriminate the dark environment. He suggested the term "dazzle".

In a criticism of the tropism theory of LOEB, the literature on photo orientation was extensively reviewed by ERHARD (1923). His attempt to interpret the results of experiments in diverging and converging beams as well as the experiments of LOEB with caterpillars mentioned earlier (p. 13) is rather confused. The fact that insects do not fly towards the sun was attributed to stimuli acting on other sense organs, like the decrease of the temperature and the disappearance of the smell of flowers at higher altitude. There the positive thermotropism and the positive chemotropism would soon overcome the positive heliotropism. But at the same time he agreed with HESS (1919) that the light gradient is the decisive factor involved in the flying towards a light source.

The article of VON BUDDENBROCK (1917) in which the flying of insects towards a lamp was attributed to compass movements performed by the animal around the artificial near light source, no doubt constitutes a land-mark in that this theory has been accepted fairly generally. It is found in almost every modern handbook of insect physiology. VON BUDDENBROCK supposed that a similar mechanism might underlie the swimming of fishes around lamps on board of fishing boats.

The light compass reaction is of great importance in animals with a permanent home like ants and bees. VON BUDDENBROCK demonstrated that it is also used by solitary insects. He observed that in direct sunlight caterpillars moved in a straight path in some given direction. But when brought into darkness the animals moved along complicated, irregular curves. The straight path is followed by the insect by maintaining a definite angle between the longitudinal axis of the body and the rays of light from the sun. This is done by keeping the stimulus from the sun in the same ommatidia for some time (VON BUDDENBROCK and SCHULZ 1933). VON BUDDENBROCK (1917) emphasized that during locomotion a new direction can be followed for one reason or another at every moment. This is a weak point with respect to his idea that also the flying of insects towards a lamp is a light compass reaction, for, in these circumstances the insect is evidently forced time and again towards the light source. Moreover, many insects walk straight on to a lamp in the dark room and not in spirals. VON BUDDENBROCK, however, observed light compass spirals in the dark room as well as light compass straight paths under natural illumination conditions. How can this be reconciled with the straight walk towards a lamp in a dark room observed so many times in insects and other animals?

Firstly different groups of insects may be involved, one group making light compass movements with regard to a lamp and another group walking straight on towards a lamp. Secondly differences in the labora-

tory illumination conditions may be involved in producing the respective results.

As will be seen both factors are involved. To begin with the second factor, we have seen previously that for producing typically straight walks towards a light source a dull black environment is very effective. In many experiments on light compass reactions, however, no special information is given as to the kind of background used. VON BUDDENBROCK (1931) pointed to the fact that the characteristic dark room illumination is very different from the normal illumination in the animal's environment, and, therefore, it would be inappropriate to the demonstration of the light compass reaction (cf. p. 86). In his own papers, however, no exact data were given with regard to the most favourable illumination conditions. In the experiments of WILLRICH (1931) in which the light compass reactions in a number of beetles were analysed, the animals walked on a *white* cardboard. These light conditions are very different from the light conditions favourable to the demonstration of the straight walking towards a light source.

As to the first factor, a number of insects that are captured by lamps at night—species of the family of the Noctuidae (Lepidoptera) for instance—show no sign of light compass reaction when placed on a table in a dark room where a lamp is burning. On the contrary, they walk straight on to the lamp.

Moreover, the negative influence of moonlight upon the trapping effect of lamps cannot be explained by the supposition that the light compass reaction is the underlying mechanism, as will be extensively discussed further on (see section III B, C and D).

As was argued in the introduction, the trapping effect of artificial light is by no means restricted to nocturnal animals. When bees are activated at night they are trapped by a lamp just like real nocturnal insects. It might be imagined that in this case a light compass reaction is involved as bees depend on this reaction in their normal diurnal life. But when bees are taken to the dark room they walk straight on towards a lamp (MINNICH 1919, URBAN 1932, BAUERS 1953b, and others).

Still, when observing nocturnal insects—and "artificial" nocturnal insects like bees—flying in the vicinity of a lamp one might get the impression that spiral movements are performed around the lamp. But on closer examination it can be observed that the curved paths are originating otherwise. Some types of such paths of animals in the vicinity of a lamp were theoretically analysed, and after that compared with actual paths under experimental conditions by BLUM (1935, 1954). He pointed to an important relation in orientation, namely the ratio of the angular (= orientation) velocity to the linear (= progression) velocity. He demonstrated that the ratio of these two velocities together with the

shape and the geometrical arrangement of the photoreceptors accounts rather satisfactorily for various paths found in actual experiments. The ratio in question determines the curvature of the paths at any given instant.

It may be imagined that in a walking insect this ratio is relatively high as compared with this ratio in the flying insect. If so, the insect on foot thus turns sharply towards a light source and then walks straight on to it, while it is conceivable that the insect on the wing flies in a more or less sharp curve towards the lamp, or, when the ratio is very low, past the lamp. BLUM stated that this kind of path is not uncommonly seen at night when insects spiral towards a source of light and then fly away from it again without any sharp change in the path. Theoretically this can be performed by the insect without changing either its linear velocity or its characteristic orientation. He emphasized that such a path might be suggestive of representing various characteristics of photo orientation such as light compass reaction, reversal of sign of orientation and the like. On the one hand BLUM pointed to the fact that the natural orientation behaviour of animals is of course far more complicated than his theoretical analysis based on some schematical assumptions would suggest. On the other hand he emphasized that in experimental conditions the organism is often brought in such a situation that its reactions will not be much more complicated than those of a mechanical model. This means that we should not consider such reactions as representative of the normal orientation behaviour of the organisms in their normally illuminated environment.

Rather confusing in this connection are the considerations of LUDWIG (1933, 1934), and LUDWIG and SCHNEIDER-HEMPEL (1954). These authors observed the walking of the caterpillars of *Lymantria dispar* towards one or two lamps in the dark room. In many cases the paths of the animals seemed to take the form of a logarithmic spiral. Originally this was attributed to a physiological asymmetry, adjusted momentarily in the central nervous system, and the reaction was called tropo-menotaxis. Moreover, and in contradistinction to the concept of light compass reaction, the light intensity gradient around the lamp would be an essential factor in initiating the walk towards the lamp. This would account for the fact that the caterpillars do not walk towards the moon, for during this movement the intensity gradient would be too weak to be perceived by the caterpillars.

It is evident that in these considerations the same mistake is made as was previously found in the work of HESS (1919), ERHARD (1923), and others. During more recent experiments (LUDWIG and SCHNEIDER-HEMPEL 1954) caterpillars with only one functional eye performed the same curved path and the conclusion was that the animals maintain a

definite angle between the impinging light rays and the direction of locomotion. The authors still maintained that the reaction was tropomeno-tactic.

LAMMERT (1926) and OEHMIG (1940) observed that caterpillars of different species walked *straight* towards a light source in a dark room. According to LAMMERT the caterpillars scorched in a candle flame. OEHMIG found that caterpillars with only one functional stemma walked straight on towards a light source. And even totally blinded caterpillars did so, though not in a straight line. Evidently dermatoptic light sense was involved in this reaction, as was the case in the experiments of PARKER (1903 a) with blinded frogs (p. 20).

Apart from differences between species, and the influence of the age of the used caterpillars, the contradictory results in the many experiments with caterpillars can probably be attributed to differences in the experimental illumination conditions, a factor which has hardly been taken into consideration by the respective authors.

A vigorous warning against unnatural illumination conditions in laboratory experiments on photo orientation was given by RUSSELL (1934) in the following words: "...bright and localized sources of light are practically unknown in nature and the apparently forced phototaxis which many nocturnal insects exhibit towards a bright lamp has something abnormal, meaningless and pathological about it. It is mainly when exposed to such unnatural conditions that the animal shows that forced and automatic response to simple physical stimuli, which according to the theory of tropisms is the basis of all behaviour. Tropisms are in fact very largely laboratory products—the outcome of exposure to artificial and unnatural stimuli". No further allusion was made as to the particular nature of these stimuli and the resulting reactions.

In an extensive review of the taxis problem, PRECHT (1942) pointed to the fact that under laboratory conditions animals frequently perform only phototactic movements with a "positive" sign, while the two other possibilities—movements with "indifferent" or "negative" sign—are relatively seldom observed. At the same time he stated that the light intensity is the only attracting or repelling factor of a light source. More recently he argued that photo orientation has some of the characteristics of orientation to temperature in that the movements of the animal are generally directed towards an indifferent zone with a preferred light intensity (PRECHT 1951, see also PRECHT *et al.* 1955). The animals would alter their preferred light intensity according to their needs of the moment. This preferendum character of photic orientation would account for the fact that night-flying insects do enter lighted open windows only in dark nights and not when the moon is shining, as only during dark nights the prevailing light intensity would be below the

preferred intensity. Moreover, the most favourable conditions for demonstrating positive phototaxis in the laboratory would be obtained by using a light source of very low intensity at a distance of some meters from the experimental animal, while negative phototaxis would result from a near light source of high intensity.

If this actually applies the night flying insects might be expected to approach the light source in a room up to a certain light intensity, the preferred intensity. The evidence at hand, however indicates abundantly that this is generally not true. In many cases the insects—and other animals—move towards a light source as far as mechanical barriers permit, and, as we have seen above, they are often forced by the light stimuli into conditions incompatible with survival. Consequently it is clear that the moving of insects—and many other animals—towards an artificial light source is not due to the search for a light intensity preferendum.

The influence of the moon on the trapping effect of light will come up for discussion when the trapping of insects, fishes and birds is discussed from a practical point of view (section III B, C and D). There it is seen again that the trapping effect of artificial light is not caused by an intensity preferendum.

The ideas of PRECHT are based in part on experiments of BAUERS (1953a) in which the walking of a number of insects in a "diffuse" light gradient (cf. p. 5) was observed. BAUERS concluded that the animals reach their respective intensity preferendum by "positive or negative light reactions". But as to what might be the nature or the mechanism of these light reactions we are left in the dark. For the rest BAUERS agrees with the ideas of PRECHT.

Diametrically opposed to the ideas of PRECHT and BAUER is the theory on photo orientation developed by VIAUD (1948, 1949, 1951a, 1951b, 1956). He maintains that the assumption of a preferendum character of photo orientation is not at all justified. According to his theory photic movements with positive sign are of a fundamentally different character as compared with movements with negative sign. These ideas are in fact based on the movements of lower animals like *Daphnia* towards the light in parallel, divergent and convergent beams. It was concluded that these movements can not be directed towards a preferred light intensity and they were considered, therefore, to be non-adaptive. The nightly attraction of Vertebrates like birds and mammals in the beams of the head lights of motor-cars in a wood are mentioned in this connection (VIAUD 1951b). He termed this type of movement "tropism" as LOEB did.

It is not clear what are the underlying mechanisms of VIAUD's tropisms. The positive movements would be elicited by dermatoptic

light sensibility at least in lower animals. VIAUD agrees with LOEB that the "tropism" is "forced". He is of opinion that it is a polarized impulsion—a directed or polarized photokinetic response identical with the attraction by light—originating from lower levels. Similar ideas were developed earlier by BOHN (1921, 1940) who thought that this polarization could be found back in tissues and even at the molecular level. The movements towards the light of posterior parts of negative phototactic Planaria's are alleged in favour of this view (VIAUD 1951a, 1951b). VIAUD admits that the positive sign of phototropism is still quite mysterious. Curiously enough the flight of insects towards a lamp is ascribed to the light compass reaction of VON BUDDENBROCK. Although animals are thus supposed always to have a primary tendency to move towards the light, this tendency could be inhibited by photonegative reactions which are considered to be essentially different in origin from positive reactions. The negative reactions are considered to be adaptive as according to VIAUD they would withdraw the animal from the injurious action of too high light intensities. They would be due to the action of the nervous system in answer to stimulation of the eyes. This type of reaction is termed "pathy".

LOEB and JENNINGS are cited by VIAUD in relation to his "tropism" and "pathy" concepts respectively, and in this way it is the old direction–intensity controversy which crops up again in his theory.

When we try to apply the theory of VIAUD to our problem, it might be suggested that the trapping effect of artificial light results from illumination conditions which are unfavourable to the adaptive action of the "pathy", thus giving free course to the always present basic phenomenon, the "tropism" towards the lamp. As is seen further on (see section V) the author is of opinion that the "tropism" towards the light is not the normal basic phenomenon of photo orientation. It is, on the contrary, an artificial reaction provoked under certain laboratory illumination conditions which are accessible to experimental analysis. Photo orientation is a complicated type of behaviour in which the animal makes use of the normal illumination conditions in its environment. It can not be divided into two mechanisms, a positive and a negative, counteracting one another in some way.

This review of laboratory experiments pertinent to our problem will be concluded with some recent considerations of THOMAS (1954). His opinion that the results of LOEB's experiments with caterpillars are due to certain details in the experimental conditions—shape of the tube, overheating—was mentioned earlier (see p. 14–15) and refuted there. Obviously THOMAS did not know how to interpret the flying of insects towards a lamp. He stated that he could not see how this could be of use to the insect and he could not but admit finally: "la question reste

en suspens". He ends with the anthropomorphic suggestion that *curiosity* might be the motivation for animals to go towards light sources!

After this statement one can not help being struck by the rather unsatisfactory circle in which theories have turned round, arriving with THOMAS at the same anthropomorphic level where they started about 75 years ago with ROMANES (p. 22).

B. THE CAPTURE OF FISHES AND OTHER AQUATIC ANIMALS WITH THE HELP OF LIGHT SOURCES

From ancient times the blinding or luring effect of light has been extensively employed by man for the catching of various animals, particularly fishes, and usually with the purpose of getting food. There are scattered reports about the use of this technique from almost all parts of the world, but predominantly from the clear waters of the tropical and subtropical zones. Actual research on the subject has been made in some countries around the Mediterranean, and in Japan, while, more recently, a general revival of interest in this technique is observed.

The development of the use of light in fisheries was reviewed by FAGE (1924), RUSSO (1928), and recently by SCHÄRFE (1953). The classical methods used in the Mediterranean and in the Adriatic were described frequently (DUGE 1913; PIFFL 1913; GAST 1918; GEISSLER 1952, and others; see also VERHEIJEN 1956). According to this method one boat shoots an encircling net around the fishes gathered in the vicinity of the lamps on board of another boat. There are similar descriptions from other parts of the world. These for the greater part old descriptions are in general so sketchy that they do not supply many data which might be useful in an attempt to analyse the physiological mechanisms underlying these techniques. Unfortunately the same holds *a fortiori* for recent descriptions of some renewed applications of light in fisheries (ANON. 1948; ANON. 1950a, 1950b; ANON. 1951; FRY 1950; LE BRETON 1952a, 1952b; YOUNG 1950). This is the more regrettable as the results of some renewed experiments in attracting fish with light are rather precarious (see p. 35–37). In the absence of any reliable description of the circumstances under which most of the more recent experiments with light were made, only a guess can be made as to the cause of the positive or negative results.

Several authors have incidentally expressed their opinion as to why or how fishes and other aquatic animals are attracted by a lamp. Their opinions may be summarized as follows: curiosity (DUGE 1913); attracted by a magic power; to eat animals already present around the lamp (GAST 1918); fascination (FAGE 1924); the fascinating, luring or blinding effect of light (HOUGH 1926); the light intensity; positive

phototaxis (GREIN 1912; RUSSO 1932, 1935; LE BRETON 1952a); almost hypnotic attraction (FRY 1950); positive phototaxis towards an optimum light intensity (SCHÄRFE 1952, 1953). A number of Japanese workers, too, are of opinion that fishes are moving towards a lamp because of the light intensity around the lamp. With other Russian workers NIKONOROV (1957) is of opinion that "according to the teaching of the academician V. P. PAVLOV, the attraction of fish by light is essentially a feeding reflex".

It is generally agreed that a moonless night or the moonless parts of a night are most favourable to the collection of fish with lamps. The paramount importance of profound darkness is demonstrated in an impressive way by the rhythm of life of the fishermen using light, as it is determined by the complicated sequence of dark and not-dark periods resulting from the interference of the times of rotation of the sun and the moon relative to the earth. Favourably dark in this sequence are only those periods in which neither the sun nor an appreciable sickle of the moon is above the horizon. As to the question why profound darkness is a *sine qua non* for the trapping effect to set in, no clear suggestions have been made. It has been suggested that the moonlight affects the concentration of fishes around a light source directly because the light source has to compete with the light of the moon and so is less efficient in attracting the animals as compared with nights without moon. Moreover, it is possible that the moonlight influences certain aspects of the animal's behaviour, for instance their general activity.

It will be seen (section III C and D) that the same problems will come up for discussion with regard to the effect of moonlight upon the nightly attraction of insects and birds by artificial light sources.

Originally light sources of low capacity (torches, etc.), held or mounted above the surface of the water, were used to attract fishes, squids, etc. (COBB 1903; HOUGH 1926). Later on more powerful sources (4000-6000 cd) were found in acetylene and petrolgas lamps (GAST 1918; RUSSO 1932; ALFONSI 1933). For research purposes a floating acetylene lamp was devised by FAGE and LEGENDRE (1923a, 1923c). Electric lamps are the most powerful sources (500-2000 watt) and they can easily be used under water. Petrolgas lamps, and to a less degree electric lamps, above the water surface have up to the present been highly favoured by the many fishermen all along the mediterranean coasts.

Three groups of relevant papers will be considered more closely. First some details about fishing with light by Italian workers; secondly a number of Japanese experiments for the greater part carried out in aquaria, and thirdly recent work with the help of echo-sounding apparatus.

During a long period (1915–1950) the Italian worker RUSSO devoted a series of papers to fishing with light. Originally he was of opinion that fishes are attracted by the plankton around a lamp (RUSSO 1915), but later on this view was abandoned. Experiments were made with the

combination of an electric underwater lamp and a kind of bow net (Russo 1915, 1917) but only small planktonic animals were captured, no fish. According to Russo, the apparatus was unsuited for capturing fish because the illuminated net could be seen by these animals. Similar results were obtained earlier by Herdman (1889) and by Grein (1912). From his observation that fishes like the Clupeids and *Scomber* are swimming nearer to the centre of the light field around a small 12 volt electric underwater lamp than *Trachurus trachurus*, *Box boops*, etc., Russo (1932) concluded that the former fishes require a higher light intensity than the latter. Recently the fact that the distance at which fishes swim around a lamp seems to vary with species and with the power of the lamp was interpreted as due to orientation towards an optimum light intensity by positive phototaxis (Schärfe 1952, 1953). Woodhead (1956) is of opinion that these movements with respect to a point source of light seem to agree very well with the observations that the movements of fishes in an experimental light gradient are restricted by the light intensity preferred by the fishes. It will be seen further on that this opinion is untenable (see section v d).

Russo (1932) observed that fishes concentrated around an electric underwater lamp disappeared after the water had become turbid as a result of a change in the direction of the stream. He stated that this was in accordance with the fishermen's experience that fishing with light is only possible in clear water (acqua nero) and not in turbid water (acqua bianca).

The behaviour of fishes and other aquatic animals in the vicinity of a light source has only seldom been described exactly. A detailed description of this behaviour was given by Monterosso (1919). Some important results of his observations around an electric underwater lamp will be rescued from oblivion. They are the more impressive as they are corroborated by recent and suggestive conclusions from observations on insects to be dealt with further on (see section iii d). Monterosso found, that the shadow of an underwater reflector is detrimental to the collecting of fishes. The fishes which arrived in this shadow disappeared out of the action radius of the lamp. Though it was an easy matter to see how busy some species of fishes—sardines for instance—were in getting their food supply from the planktonic organisms around the lamp, he emphasized that other species arrived near the lamp independently of the presence of plankton. Particularly interesting is his description of the obviously abnormal behaviour of many fishes and other animals around the lamp. Some features of this peculiar behaviour were: swimming close to the surface, sometimes standing almost motionless, touching the lamp or suddenly dashing violently against it; jumping out of the water; floating at the surface, sometimes with the

body bent horse shoe. Frequently fishes seemed to be stupefied or dazzled and they were easily captured with a small net, as was already stated by DE MONACO (1895).

More recently WESTENBERG (1951) is of opinion that the general behaviour of fishes under a lamp seems rather to suggest a general lack of orientation. From the behaviour of fishes, squids etc. as observed during the nightly sardine fishery between the island of Ischia and the Italian coast, the author likewise got the impression that this behaviour was the result of a certain degree of disorientation (VERHEIJEN 1956; cf. section IV B of the present paper).

Japanese workers have recently tried to analyse the influence of various factors such as intensity, colour, moon, etc. on the "gathering rates" of lamps as they call it (KAWAMOTO and co-workers 1951, 1952, 1954 and 1955; SASAKI 1950a; OZAKI 1951, 1952; MAEDA 1955). Though they do not give their opinion as to the mechanism of this gathering of fish around a lamp, it is obvious from their experiments that their starting-point is that the fishes swim towards a preferred intensity and colour in the light field around the lamp. Most of their experiments were carried out in rather small tanks with two or more light sources of different intensity or colour. The numbers of fishes observed beneath each lamp under several conditions were analysed statistically with the purpose of finding out which intensities and colours are the most effective in "gathering" fish.

Although it will not fully be realized until the end of the present paper that such experiments with fishes are inapt for the solution of the problem in question, it can easily be demonstrated now that the gathering of fishes near lamps as observed in aquaria should not be identified with the nightly concentration of fishes around a fishing lamp. First very young fishes were used in the above mentioned experiments. It is generally accepted, however, that young fishes prefer higher light intensities as compared with adult fishes of the same species. Consequently, an intensity preferendum found in experiments with young fishes can not be used to explain the behaviour of adult fishes around a lamp as observed during actual fishing. Secondly, the photo preferendum of fishes and many other animals generally shows a diurnal rhythm. Some fishes that are active in the daytime prefer relatively high intensities during this period, while during the night when there is a considerable decrease of activity, their preferendum is shifted to lower intensities. KAWAMOTO and KONISHI (1955) observed this phenomenon in some of their young experimental fishes: during the daytime the fishes gathered near the lamp, whereas during the night they avoided the illuminated area of the tank as far as possible (*Girella punctata* Gray), or even fell asleep in the darkest area of the tank (*Rudinarius ercodes*

Jordan and Fowler). But it is just during this period that fishes must be "gathered" with the aid of light. Obviously the incompatibility of these facts has been overlooked. Thirdly, no mention is made of any of the features which were described earlier as being characteristic for the abnormal behaviour of fishes around a lamp at sea.

On closer examination the possibility that an intensity preferendum or optimum is involved in the concentration of fishes and other aquatic animals around a lamp becomes more and more improbable. First, the number and variety of marine organisms that are concentrated around light sources is enormous: protozoans, annelids, crustaceans, cephalopods and fishes have been observed in the vicinity of a lamp burning under or above the surface of the water (see for instance DE MONACO 1895; FAGE et LÉGENDRE 1927; WOODS 1952). Secondly species of animals captured in former times with weak light sources are not less well captured with powerful light sources. On the contrary, there has been a general increase of catches parallel to the use of more powerful light sources.

The opinion that an intensity preferendum is involved in the concentration of fishes would imply that the intensity of illumination around the most powerful electric lamps used in the fisheries would still be below the intensity preferendum of the concentrated fishes. This is the more improbable when it is realized that some species of the clupeids are the pièce de résistance of light fishery in many parts of the world. The fact, however, that some clupeids—herrings, sardines, anchovies—are captured in the daytime with bottom-nets and during the night with drift-nets near the surface, has long indicated a diurnal vertical migration of these fishes (LISSNER 1925; LE GALL 1928; RUSSO 1935, 1950; BALLS 1951). The elucidation of the mechanisms of the diurnal vertical migration of fishes has been the object of many observations of fishes in their natural habitat, more recently with the help of the echo sounding technique and by divers equipped with an aqualung.

The diurnal vertical migration of some freshwater fishes was observed in lakes by LASSLEBEN (1951, 1952), SCHÄRFE (1951), HASLER and VILLEMONTE (1953), and others. The behaviour of the blue perch *(Perca flavescens)* as observed by HASLER and VILLEMONTE is interesting in so far as, in all probability, it is characteristic of the behaviour of fishes that are not likely to concentrate around light sources. In the daytime the perch were observed in schools in deep water at a depth of about 10 m. After sundown no schools were found; instead, the individual perch were observed dispersed along the bottom of a less deep shelf and resting with their pectoral fins touching the sand. They fled away towards the dark when struck by the light of a lamp while "sleeping" at the bottom. This behaviour of the perch is reminiscent of that of *Rudinarius ercodes* as observed by KAWAMOTO and KONISHI (1955).

Quite different in this respect is the behaviour of clupeids. RUSSELL (1926–27, 1928, 1929–30) was one of the first to analyse the apparent

paramount importance of the light intensity in controlling the vertical distribution of the pelagic young of clupeids. During the night or in the daytime during dull weather they were found higher in the water than on sunny days. Recently similar observations were made with echo sounding apparatus by De Boer (1950b) on young herrings of about 10 cm long. An attempt to analyse the vertical migration of herring larvae was made by Woodhead and Woodhead (1955). Fullgrown herrings were recorded more than once at a depth of 100 m or still deeper in the daytime and they could be seen to rise to lesser depths in the gloaming (Runnström 1941, and others).

Though Krefft and Schüler (1951) suggest that feeding might play an important role in inducing the vertical migration of herrings—prey animals like *Calanus finmarchicus* actually perform about the same migrations—Richardson (1952a) observed that the diurnal migration takes place in both feeding and non-feeding herrings and sprats, and the increasing light intensity was thought to be the operative cause for the downward movement at daybreak. From an analysis of the gut content of herrings captured at different times of the day in the Western North Sea, Lissner (1925) and Mužinić (1931) concluded that the herring feeds predominantly during twilight but neither in bright daylight nor during profound darkness. Experiments in aquaria (Battle *et al.* 1936; Johnson 1940; Verheijen 1953) have revealed that herrings are still active and that they feed by sight at light intensities down to the order of moonlight.

Echo sounding observations on the influence of artificial light upon the vertical distribution of clupeids and some other fishes have led to rather contradictory results. According to de Boer (1950a, 1950b) young herrings of about 10 cm long showed a marked downward movement upon being lighted by a searchlight, while a school of larger herrings dispersed. After a searchlight was switched on, Hodgson and Richardson (1949) observed that a school of pilchards at first descended but then ascended to a higher level. When the light was turned off the school descended to the former level. After a searchlight (100 watt) was switched on, Krefft and Schubert (1950) observed small young fishes, but in addition herring, mackerel and garfish (*Belone*) with an echo sounding unit as well as by sight at the surface. After the lamp was switched off the fish disappeared. Schüller and Krefft (1951) found, however, that a searchlight (500 watt) at the surface as well as an underwaterlamp (40 watt) did frighten herring away. Only young mackerel and some other young fishes were observed swimming towards the light. This was ascribed to the generally observed fact that young fishes are positively phototactic while the older ones are negatively phototactic.

The author, however, witnessed the nightly capture of about 20 cm long specimens of the herring-like clupeid *Clupea aurita* Gthr., together with sardines and anchovies, with the help of petrolgas lamps in the Mediterranean near the island of Ischia.

RICHARDSON (1952a, 1952b) directed a searchlight on to shoals of herrings and pilchards in the Cornish waters in order to gain some insight into the action of light upon the vertical migration and to make some experiments in attraction with light. Herring shoals avoided the light of the 60 watt searchlight. As soon as the light was switched on, the shoal was seen to descend, while the fish rose to their former level as soon as the light was switched off. When the searchlight was directed on to a shoal of pilchards, the top of the shoal descended for a few seconds, but then, however, started to move towards the surface; the fish could be seen jumping out of the sea in the searchlight beam.

RICHARDSON agrees with others that in the normal diurnal vertical migration the downward movement of shoals of herring towards daybreak is associated with the increase in light intensity and that the fish remain within a certain light intensity during the day. The level towards which the fish rise during the night, however, is regarded to be restricted by the gas bladder[1]. This supposition is derived from JONES (1952) who found this in the perch. But it seems not justified to deduce the behaviour of clupeids from that of the physoclist *Perca fluviatilis* as observed by JONES. For we have found in laboratory experiments that the well known gas-spitting reflex of the other physostomes is substituted by the release of gas bubbles through the posterior opening of the gas bladder in the sprat (VERHEIJEN 1953), the herring and the sardine (VERHEIJEN 1956). In the meanwhile we observed that in the anchovy, where this posterior gas bladder opening is absent, the normal gas-spitting reflex of the physostomes is evoked by pressure lowering. Moreover, the air bubbles rising ahead of ascending clupeids are used in many parts of the world as "fishermen's signs" from which information is gathered about the position of the shoals. Consequently the gas bladder is not likely to be a restricting factor in the nightly ascent of clupeids. It seems rather more plausible to regard the light intensity as an important regulating factor, as it is assumed to be in the descent, though other internal and external factors like age, food, temperature, etc. are likely to modulate both ascent and descent according to needs.

Although much additional research is yet to be accomplished on this complicated problem, the results thus far point towards the theory developed by BALLS (1951) for the herring, according to which the diurnal vertical migration is photophobically driven. In other words the clupeids in question—herring, sardine, anchovy—have a low light intensity preferendum.

With a 500 watt lamp WOODS (1952) collected many species of fishes, among others *Halieutichthys aculeatus* Mitchill. This species is usually captured in the trawl at depths of 30–200 m, and from aquarium observations the species is known to be active only during the night. During the day it rests buried in the sand. It seems reasonable to conclude that this fish, too, prefers low light intensities. Consequently it is not clear how the concentration of such fishes around a powerful light

[1] The term "gas bladder" comes from WOODLAND (1911) who stated with justice that "the terms 'swim bladder' and 'air bladder' are inappropriate, since fishes do not use this apparatus for swimming and it does not contain air".

source could be understood on the basis of the intensity preferendum theory.

When reviewing the more recent papers on experiments with light, we cannot help getting the impression that workers have not been aware of the fact that the two different objects which they have in view—the elucidation of the part played by the natural illumination conditions in the vertical migration of fish on the one hand, and on the other hand, an understanding of the attraction of fishes by lamps – require the designing of different experiments (cf. section v).

A number of experiments, carried out to find the light colours attracting most fishes, have led to contradictory results.

KAWAMOTO and KONISHI (1952) found in experiments in tanks that green and blue "produce the greatest fish-gathering rates". KURIEN *et al.* (1952) collected most fishes with green, blue and—somewhat surprisingly—with red light. SASAKI (1950a) obtained rather indistinct results in collecting fish with lights of different colours. MAEDA (1955) could not demonstrate any influence of the light colour upon the behaviour of fish in experiments in tanks.

In experiments with schooling young fishes (*Girella punctata* G. and *Mugil cephalus* L.) OZAKI (1951, 1952) found a preference for green and blue light when he used a shoal of these fishes. When using one fish, however, no colour preference was found. In order to get some insight into these results it might be imagined that the main activity of one isolated shoal fish is to seek for others (KEENLEYSIDE 1955). Less important factors in their environment like the colour or the intensity of light are disregarded until two or more fishes have met together and formed a shoal.

A number of these experiments with coloured light are open to criticism in similar respect like those with white light pictured before (see p. 33–34).

Lastly, one additional factor that seems to play an important part in fishing with light will be put forward. The technique is generally applied in waters of considerable depth. When clupeids are involved it seems reasonable to suppose that these fishes are captured during the the night at the surface at places where, during the daytime, they can shy away from the strong light into water depths of at least some tens of meters. Moreover, these depths would secure against too strong an illumination of the bottom by the artificial light source. The importance of this circumstance can only be rated at its true value further on (see section IV B and section V). That this factor actually plays a part can be derived from facts like the following. In Lembeh Strait, in the Indonesian waters, anchovies, sardines and spratelloids are used as live bait in fishing for tunas. These bait fishes are gathered during the night by light and after that guided towards the shore. WESTENBERG (1951) stated that the slope of the beach should not be too gentle and the sand should be black as the fish are not likely to follow when the bottom becomes visible in the shine of the lamp.

Notwithstanding the general lack of insight into the trapping effect

of artificial light upon fishes, some new fishing techniques that are based on this phenomenon were recently developed.

There are some short reports of Russian successes in fishing with light in the Caspian Sea (Anon. 1950b; Anon. 1951; Le Breton 1952a, 1952b). A rather successful technique was developed by Sasaki (1950a). Along a string of floating electric lamps that are extinguished one after another, fishes are guided into a trap net. A very curious and successful fishing technique was recently developed in the Caspian Sea (Nikonorov 1957). Clupeids are attracted by an underwaterlamp and then sucked on board with a pump. This technique has assumed enormous proportions.

When reviewing the widely divergent and often contradictory experimental results and opinions with regard to the fishery with light it might seem a hopeless task to gather the conditions required for an optimal "attraction" of fish from the available data. However, when the foregoing data and considerations are combined with those given previously under A, the following conditions seem to meet these requirements: *a.* an isolated light source; *b.* profound darkness (no moon); *c.* clear water; *d.* no illuminated environment or background (bottom, nets, etc.); *e.* a light field without shadows from near objects; and *f.* fishes that are active at places and during periods necessary for the just mentioned conditions.

As to the question *why* fishes are being attracted by light under these conditions, we are of opinion that no "normal" or "innate" intensity preferendum is involved. Fishes are "forced" to concentrate around an artificial light source in a way that will be elucidated in section V.

C. BIRDS AND LIGHTHOUSES

Birds represent a second group of animals which are victimized in mass by artificial sources of light, especially by lighthouses and lightships. Well-known as the phenomenon as such may be, little experimental work appears to have been done in order to explain it.

A number of authors observed the behaviour of birds in the vicinity of lighthouses. In his extensive work, Clark (1912) has provided us with some valuable data. In Vol. 1 of this work he states (p. 284–285): "The lantern of a light-station... is simply a decoy... that would only 'work' under peculiar conditions, which are dependent upon the amount of moisture (rain, haze, cloud) present in the atmosphere. When moisture is disseminated through the air as a liquid in a state of minute subdivision, the mixture becomes more or less opaque, and the powerful beams from the lantern then become conspicuous to a very remarkable degree, and exert extraordinary attractive powers over the

migrants that pass within the sphere of their influence". In Vol. II the "favourable" conditions were described more closely as follows (p. 24): "...the existence in the atmosphere of moisture not necessarily in the form of rain or haze, but actually present, though not visible, on dark starless nights". But he stated also: "I have seen them (the birds) in great abundance at the lanterns when I could make out neighbouring lights that were ten miles or more distant", and "on the other hand, there was not a single instance of migrants visiting the light when the night was bright and starlit or the moon visible". He observed further (Vol. I p. 286, 287) that "The birds which struck the lantern did so after travelling up the beams of light...". According to his observations "the starling was the most susceptible subject present; and this clever bird became, under the sway of the lantern, not only a complete fool, but a seemingly willing sacrifice... Great numbers were removed from the lantern and cast over into the darkness below; but many of them immediately returned". From these descriptions two facts become obvious: *a.* the flying of the birds against the lantern has a "forced" character, and *b.* the phenomenon appears most clearly during dark cloudy nights, which must be largely nights with a high degree of moisture in the atmosphere.

VERWEY (1924 28) realized in which ways birds are approaching a lighthouse. There are three possibilities: first the birds may reach the tower while flying above the rays radiating from the lantern; secondly they may do so while flying below the rays; and thirdly they may fly in the plane of the rays of light. Depending upon the general illumination conditions and the colour of the tower there is a reasonable chance in the first two cases, that parts of the tower are perceived in time and that the bird succeeds in evading those parts of the tower. The same holds for the third case, when, at least, the bird reaches the lantern between two beams. When, however, the lantern is reached while the bird flies in a beam, the animal will strike the lantern, generally with fatal results. In this connection VERWEY threw doubt upon the alleged useful effect of the protection ladders that are, moreover, used almost exclusively by the starling (*Sturnus vulgaris*). He stated that the illumination of a lighthouse building and its immediate environment seems to protect the birds to some extent.

For a further consideration of the influence of the general illumination conditions upon the attraction of birds by lighthouses and more especially the influence of moonlight upon this phenomenon, the influence of moonlight upon the nightly migration as such can not be left out of consideration. DÖRR (1932) suggested that there is in certain parts of Europe a lunar influence on the data of arrival of migrating birds. He concluded that migration is maximal during nights with

moonlight. BRETSCHER (1934), however, arrived at the conclusion that an influence of the moon upon the nightly migration could not be demonstrated. DROST (1935) observed that the lighthouse of Heligoland attracted masses of birds around new moon, and he concluded that during these periods great numbers of nightly migrants are on their way. With the moon shining the attracting power of the lighthouse disappeared. WAGNER (1937) observed large concentrations of birds around lighthouses when in the course of the night the sky became cloudy after an initial bright period. He attached much importance to the invisibility of the earth or the sea under illumination conditions during which the attraction of birds by lighthouses or lightships is very strong.

The fact that lighthouses in the neighbourhood of strongly illuminated harbours are considerably less attractive to birds in comparison with lighthouses on isolated islands etc., fits well into the general picture.

Birds can be captured with light. Some old techniques were described by HOUGH (1926). HILPRECHT (1937) captured starlings (*Sturnus vulgaris* L.) for banding purposes with the aid of a large bow-net equipped with a headlight of a car.

TORNIELLI (1951) gives an account of the behaviour of birds attracted to a large fire. This fire originated from a methane gas well. The flame was some tens of metres high and lasted about two months. He observed that the birds deviated from their direction of flight, and that they were forced to move towards the flame in an uncoordinated and often fatal flight. Birds with burned wings were regularly found around the fire. TORNIELLI is of opinion that the phenomenon is due to "an instinctive curiosity of the birds which stimulates them to approach the light which relieves the monotony of the darkness, and which, thereupon, makes them lose orientation by its glaring effect, and forces them to rotate around the light during a long time".

When reviewing the available data and theories in sober reason the inevitable conclusion will be that there has not been made much progress in the elucidation of the attraction of birds by lighthouses since the excellent and impressive descriptions of CLARK.

Some further data pertinent to the problem may be derived from casual observations made by KRAMER (1949), SAUER (1957), and SAUER and SAUER (1955) during studies of the nightly orientation of migrating birds. The experimental cage used by SAUER and SAUER allowed the birds to observe a part of the sky at an angle of about 68°. When stars were visible, the birds (*Sylvia borin* and *Sylvia atricapilla*) were orientated in their normal migration direction. However, when the moon became visible above the opaque side walls of the cage, the birds got desorientated and turned "positively phototactic" towards the moon. When in a dark room light radiated from one side a similar phenomenon was

observed: the birds behaved "positively phototactic" towards the light just like they did with respect to the moon. KRAMER, on the other hand, observed no influence of the moon upon the direction of orientation of the cagelings used in his experiments (*Lanius collurio*, *Sylvia atricapilla*).

The difference in the behaviour of the birds in these two groups of experiments is no doubt due to some differences in the design of the experimental cage used by the respective authors. The cage used by KRAMER offered the experimental birds an unobstructed view in all directions. The cage used by SAUER and SAUER, on the other hand, was equipped with opaque side walls, leaving free a visual field to the sky of only 68°. It will be seen in section IV C that under similar experimental illumination conditions birds are forced to turn towards a light source.

VISSER and RADEMAKER (1935) studied the photic reactions of the pigeon after ablation of the cerebrum. The variability of the experimental conditions, which, moreover, were not exactly described, makes it difficult to evaluate the results of these experiments. The overall impression of VISSER and RADEMAKER seems to have been that the birds were "positively phototropic" in answer to weak light stimuli, and "negatively phototropic" in answer to strong light stimuli. The walking or flying towards and around a lamp, for instance, was considered to be the result of an alternation of "positively and negatively phototropic" reactions in answer to a light intensity, which varied with the distance between bird and lamp. It is clear that the dashing of birds against the lantern of a lighthouse with its enormous light intensity can not be explained along these lines of thought.

VIAUD and MARX (1948a, 1948b, 1948c) investigated the walking of normal pigeons and pigeons without cerebrum towards a light source in a black tunnel. The reactions were essentially the same before and after operation. The attraction of the pigeons by light was thought to depend, at least in part, on a "need to use the visual function" ("besoin d'exercice de la fonction visuelle") which is proved by the augmentation of the speed of locomotion and the degree of attraction after a sufficient long stay in the dark. This idea is based upon the assumption that in the dark the activity of the birds decreases continuously for at least about 50 hours. It might be objected, however, that the increase in the "attraction" of the pigeons which becomes apparent, after the birds have been in the dark for 10–20 hours (see the curves given by VIAUD and MARX 1948c), is caused by the fact that the pigeons have slept their fill and that they are becoming active again. The possibility that a 24 hours activity rhythm is involved in these results should be fully taken into account, for it is abundantly clear from the literature that the rhythmicity of many a physiological process has basically an endogeneous character and that it persists for a consider-

able time after elimination of any environmental influence (see for instance ASCHOFF 1954; HARKER 1958).

In conclusion, we may say that the data presented in the respective papers cited above, have not been collected or analysed in a way which would be adequate to demonstrate the mechanisms underlying the attraction of birds by artificial light sources. It will be clear, however, that what little is known about the attraction of birds by lighthouses and other artificial light sources, is in general consistent with the data obtained from the literature on fishes: birds are attracted most strongly by an isolated light source in a dark environment during cloudy nights without moon or stars.

D. THE CAPTURE OF INSECTS WITH THE HELP OF LIGHT SOURCES

The concentration of insects around a lamp has been experienced as an almost inevitable inconvenience whenever man tried to dispel the nightly darkness from his surroundings. Entomologists, however, have gratefully utilized this phenomenon to collect nocturnal insects. Scattered through the literature there are occasional references to the use of light traps for pest control but it seems that this technique is only useful under certain conditions. Recently the interest in the light trap technique has strongly increased as it appeared to be of considerable value in faunistical, phenological, and other branches of insect research.

The efficiency with which insects are attracted by light can be concluded from the tremendous numbers that are captured during one night and from the fact that there are reports of the capture of species of insects in territories where these species were considered to be very scarce or where they had never been found before by other sampling techniques.

The greater part of the extensive literature on the subject is of no use to us as the exact conditions under which the insects were collected were not given, whereas all attention was concentrated on the species collected.

There are, however, some data as to the conditions most favourable for catching insects that fit in with similar data derived from the fishery and from the lighthouse problem.

It is generally agreed that on nights of full moon only few insects are captured in light traps, and that the periods of maximal catch correspond more or less to the no-moon periods.

WILLIAMS (1936) pointed out that low catches on moonlit nights need not necessarily be caused by the light of the moon, but might be due to the lower temperature usually occurring during such nights in comparison with cloudy nights. An extensive

statistical study of the question was made and he could demonstrate that the moonlight is indeed the factor involved. The captures of insects showed a lunar periodicity with a minimum at full moon and a maximum at or just after no-moon. This was particularly distinct in the family Noctuidae of the Lepidoptera which have their maximum activity round midnight, but was far less marked in certain other groups, such as Coleoptera and Jassidae which fly chiefly at dusk and dawn. WILLIAMS could not establish whether the effect of the moon light is due to a reduction of the activity of the insect, or to the circumstance that the light of the trap has to compete with the light of the moon, and so is less efficient in attracting insects.

No doubt the admirable work of ROBINSON and ROBINSON (1950) and ROBINSON (1952) is of fundamental importance as a guide to an understanding of the attraction mechanism of lamps. They observed the behaviour of insects in the vicinity of a light trap placed in the open field. Apparently unaware of the fact that so many workers have previously tried to throw light upon this problem, and unprejudiced by the respective theoretical considerations of these workers, ROBINSON and ROBINSON at once succeeded in going to the very root of the matter.

Considering that very high concentrations of powerful light sources of considerable surface brightness occur in suburbs of towns bordering on rural areas with insect populations without apparent congregations of insects around these light sources, while important congregations may be observed in closely adjacent areas around isolated lamps of comparatively low power, they arrived at the important conclusion that "*it is some special property of an isolated lamp which attracts insects*". A light source was envisaged as the centre of a "sphere of dazzle" surrounded by an "annulus of repulsion". When approaching an isolated light source, night-flying insects are at first repelled by the annulus of repulsion, but when their reaction is insufficient in some way, "their normal flight is thereupon involuntarily diverted so as to cause them to pass near the lamp". This deflection is suggested to be caused by "an imbalance set up by the failure of optical adjustment to the high light contrast" between the high surface brightness of the lamp and the dark background.

By a systematical analysis of the factors "power" and "surface brightness" of the light source it was found that an increase of power produced an increase of the area affected, and, therefore, an increase in the total number of insects taken, with a tendency towards a decrease in the number of species taken. An increase in surface brightness, on the other hand, produced an increase in the number of species. ROBINSON and ROBINSON inferred from their experiments that the ideal light source would be one of infinitely high power and infinitely small size.

Although the hypothetical character of these considerations can not be denied, it will be seen further on that they do fit in very well with the theory on the attraction mechanisms of artificial light to be developed in section V.

The "Robinson trap" which was designed in accordance with the theory of ROBINSON and ROBINSON shows a number of features the importance of which is emphasized when it is realized that these features are identical with the conditions which are optimal for collecting fish (p. 38), and fatal to migrating birds with respect to lighthouses (p. 42). In the Robinson trap the light source (a mercury vapour lamp) is mounted in such a way above the trap that there are no shadows in the light field. When a shadow cone is present –as is the case above the roof of other traps like the well known Rothamsted design (Rothamsted Experimental Station, Harpenden, England) or the Minnesota trap – certain groups of insects, for instance the fast flying Lepidoptera, escape from the trap when flying into this shadow. The Robinson trap is placed in such a way that almost no light falls upon the environment or the near background.

In spite of the fundamental importance of their experiments, the work of ROBINSON and ROBINSON has not penetrated beyond the sphere of applied entomology, where the Robinson trap is already a standard apparatus.

As will be clear, there is no room in the ideas of ROBINSON and ROBINSON for conceptions like "positive phototaxis", "optimal intensity", "preference" and the like which are still *en vogue* among workers in this and related fields. The inadequacy of such terms is again illustrated by the peculiar behaviour of desert locusts in the light beam of the head lights of a motor car as described accurately by RAINEY and ASHALL (1953). The trapping effect of the light beam was thought to result from the loss of stability in flight experienced by locust entering the illuminated area.

Several investigators have reported studies to determine the wave lengths most effective in collecting insects (COLLINS and MACHADO 1943; FROST 1953, 1954, 1955; GLICK and HOLLINGSWORTH 1955; WILLIAMS 1951 and WILLIAMS *et al.* 1955; V.D. POL 1956). Some insects are distinctly more strongly attracted by wavelengths at the ultra-violet end of the spectrum, so called "black light" as compared with "human visible" white light. This is not surprising in so far as it has abundantly been demonstrated that noctuid moths and other insects have a high sensitivity in the violet and ultra-violet part of the spectrum (see VON BUDDENBROCK 1952; WULFF 1956). The paramount importance of the ultra-violet part of the spectrum has recently been demonstrated in some insects (DAUMER 1956; WALTHER and DODT 1957).

It will be clear from the previous considerations and especially from the work of ROBINSON and ROBINSON, that the concentration of insects around light sources emitting different wave-lengths has nothing to do with the normal or innate proclivity of certain insects to fly towards and alight on distinctly coloured surfaces (flowers, etc.). Therefore, the literature on this phenomenon—"colour tropism" as it has once been termed rather inadequately—will be left out of consideration.

Judging from the previous data and considerations, the following conditions seem to be optimal for collecting night-flying insects with light

sources: *a.* an isolated source of light; *b.* wave lengths to which the insects are most sensitive; *c.* dark nights without moon; *d.* a dark environment; and *e.* a light field without shadows from near objects.

The picture which has emerged from the discussions of the data presented in section III A, B, C and D, seems to indicate rather clearly which illumination conditions do elicit the characteristic disorientated behaviour resulting in the concentration of widely separated species of animals around an artificial light source.

Analysis of these data has led to a working hypothesis according to which a certain reduction of the illumination intensity of the environment interferes with normal photo orientation, resulting in a drift towards the light source.

IV. EXPERIMENTS

The experiments to be described in this section were devised in order to investigate whether an adequate screening of the light scattered from the sky and the elimination of the reflection of light by the environment, really results in a disorientated drift of the animals towards the light source, even when this is the natural light source, the sun, and whether widely separated species of animals can be forced to move towards artificial light sources under similar experimental illumination conditions. More detailed qualitative or quantitative properties of the experimental light fields, which might be of importance in a further analysis of the movements of each single species used in the experiments, are left out of consideration as being beyond the scope of this paper.

A. EXPERIMENTS WITH BEES

The experiments on the photic reaction of insects were carried out with bees. At first sight the choice of this experimental animal might seem a curious one. But the mechanisms which determine the concentration of animals around artificial light sources are so fundamental that they may be looked for in species which are normally not active at night and which are, consequently, almost never collected in light traps (cf. p. 2).

This can also be said of bees as follows from the fact that several investigators have experienced great difficulties in trying to keep a colony of honey bees in a restricted and artificially illuminated space (BELING 1929, 1931; DRABATY 1931; WAHL 1932; CHAUVIN 1953). As soon as the bees left their hive they immediately flew towards the lamps which illuminated the experimental room, and they remained circling around these lamps until they fell down from exhaustion. LECOMTE (1955) found that after sundown artificial light sources acted as a trap upon bees kept in a greenhouse, the bees flying round the lamps and being unable to return to their hive. Recently,

RENNER (1955, 1957) succeeded in keeping bees in a restricted and artificially illuminated space by using diffuse illumination by means of a ceiling of transparent paper behind which lamps were burning. The walls of the experimental room were white.

Moreover, insects like bees have certain advantages over the first nocturnal insect. For in order to designate their movements under certain experimental illumination conditions as "abnormal", it is very profitable that it is possible to compare the observed movements with the normal movements that were to be expected under natural illumination conditions, for instance when they are conditioned to collect sugar syrup from a feeder, or when they are liberated in "strange" territory (BETHE's "box experiment", 1898).

Experiments were made with bees in a rectangular test tunnel placed outdoors and illuminated by the sun (see fig. 2). This tunnel was made

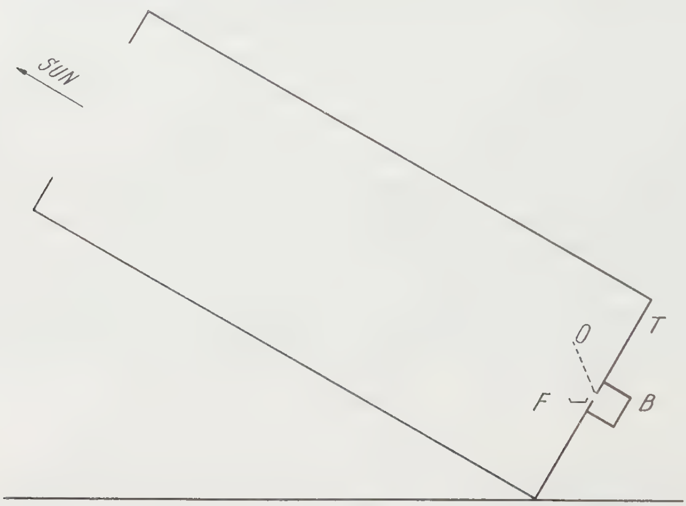


Fig. 2. Diagram of test tunnel directed towards the sun. T test tunnel; O opening through which the bees were released in the experiments of series A 1 (p. 47 ff.); F feeder to which the bees were conditioned in the experiments of series A 2 (p. 49 ff.); B box from which the bees were liberated in the experiments of series A 3 (p. 51 ff.).

of hard board and was 120 by 120 cm and 300 cm long. The inner surface of the test tunnel was coated with a flat white or black paint. In some experiments the black paint had an additional absorbing capacity in the ultraviolet region of the spectrum. The results of these experiments were identical to those in which "normal" black paint was used, and therefore only two types of inner surfaces will be distinguished: white and black ones. The tunnel was mounted outdoors in such a way that it could be directed with its long axis parallel to the sun rays.

During the experiments the position of the tunnel was corrected about every ten minutes. The wall of the tunnel directed towards the light source (the sun) will be referred to as the front wall of the tunnel, the opposite side as the background. The other four walls will be termed floor, ceiling and side walls respectively. In most experiments the light of the sun entered the tunnel through a circular orifice in the front wall, 80 cm in diameter. In this way a parallel beam penetrated the tunnel and illuminated a circular area, also 80 cm in diameter, of the background. Floor, ceiling and side walls were shadowed by the remaining parts of the front wall. As far as possible experiments were carried out during periods without clouds and at air temperatures above 20° C.

1. *Release of bees within the test tunnel, placed in familiar territory*

During this series of experiments bees were released one by one in the test tunnel with either black or white walls through an opening (diam. 1 cm) in the centre of the background. The bees were foragers coming home or departing. They were captured with a catching tube (for a description of this instrument see De Groot 1953) at the entrance of their hive in the apiary of the laboratory close to the tunnel, and at once used in the experiment. The behaviour of the bees was observed through the orifice in the front wall of the tunnel. Care was taken that, while standing on a ladder, the observer cast no shadow in the tunnel. With the aid of a stopwatch it was determined how much time elapsed between the flying of a given bee from the opening in the background and the moment at which this bee passed the plane of the orifice in the front wall. This time was called the flight duration. It was measured with an accuracy of one second. Sometimes a bee did not start flying immediately upon appearing in the opening, but kept quiet or walked around on the background for some seconds before she flew away. These seconds were not counted in. The flight duration of most of the bees was less than 25 seconds. When a given bee remained flying in the tunnel for 25 or more seconds, this bee was no longer observed and this animal was classed in the "group of bees with a flight duration of 25 or more seconds". In the tunnel with black walls a number of bees span round, thereby dashing audibly against the walls. After that they sometimes fell to the floor of the tunnel and disappeared out of sight in the shadow for some moments. But soon they started flying again and then their flight duration could be established.

The results of two experiments, one carried out with hundred bees in a test tunnel with black walls and the other in a test tunnel with white walls, are presented graphically in fig. 3. It will be seen from this graphs that there is a marked difference between the flight times of the bees under the two experimental illumination conditions. There is one

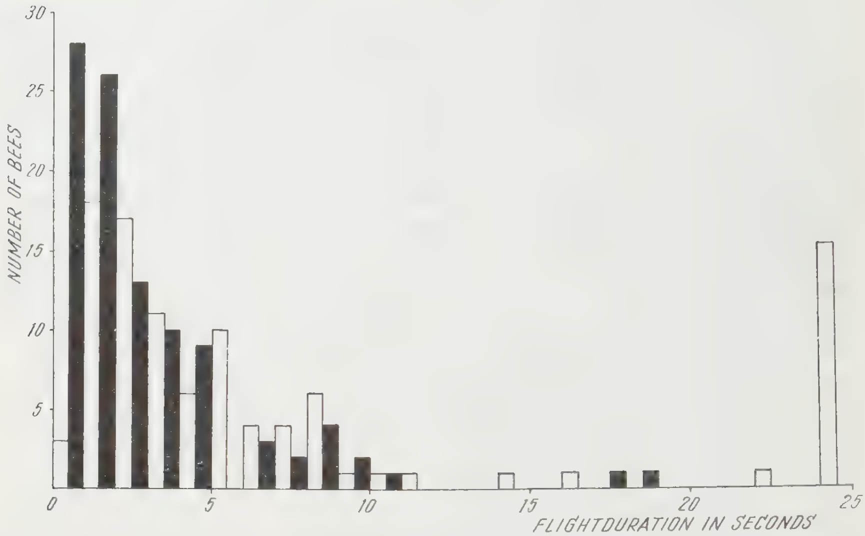


Fig. 3. Flight duration of a hundred bees released in the white (open columns) and black test tunnel (black columns) respectively.

group among the "black tunnel" bees—namely the bees flying out of the tunnel within one second—which is almost absent among the "white tunnel" bees, whereas the group of bees with a flight duration of 25 or more seconds—represented in fig. 3 by the group with a flight duration of 25 seconds—which is rather large in the "white tunnel" bees, is absent among the "black tunnel" bees.

The behaviour of the majority of the bees released in the white tunnel was highly reminiscent of the normal behaviour of bees released somewhere in the neighbourhood of their hive. After having left the opening in the background of the tunnel a given bee made some orientating flights in the vicinity of the opening and then flew out of the tunnel. Sometimes the orientating flight was continued for several seconds and in the meantime a bee might alight more than once on the intensely illuminated white background before definitively flying out of the tunnel. These intermezzos were counted in while determining the flight duration. Other bees flew out of the tunnel within one or two seconds along an almost rectilinear path and without observable orientating flight.

Quite different was the behaviour of the majority of the bees released in the black tunnel. A number of them was out of the tunnel within a second (and some even within half a second), as if they were shot from the opening in the background. Sometimes the orifice in the front wall was missed and the bee in question could be heard dashing with violence against the part of the front wall which bounded the orifice.

Other bees made wild spinning random movements, while they flew at high speed and struck the walls or the floor, obviously being unable to steer or to keep equilibrium. This behaviour is highly reminiscent of the behaviour of bees flying in a dark room in the vicinity of a lamp. There was an abrupt change in behaviour at the moment when a bee passed the orifice in the front wall. As soon as the bee was outside the tunnel, the normal flight was restored, and sometimes some orientation flights were made around the orifice of the tunnel before the animal flew away definitively.

It might be objected that in these experiments the behaviour of the bees was strongly influenced by the fact that they were captured immediately before their introduction into the test tunnel. Consequently, the described behaviour patterns might be interpreted as due to a "positive phototactic escape reaction" (however see p. 23). Moreover both returning and departing foragers were used indiscriminately, which might be thought objectionable because they might behave differently. Therefore some further series of experiments were carried out in which bees were decoyed in the tunnel by a feeder with sugar syrup.

2. *Experiments with bees conditioned to a feeder*

In these experiments use was made of our detailed knowledge of the behaviour of bees at a feeding place. Opfinger (1931) demonstrated that the orientation flight after leaving a feeder provides the bee with information about the location of the feeding place in relation to the optical marks in the environment. Other features of the feeding place like the form, the colour or the odour are noticed during the approach flight (Opfinger 1931, 1949). As these latter qualities were identical during the following experiments, they were left out of consideration.

Bees were trained to collect sugar syrup from a feeder attached to the background of the test tunnel in such a way that the feeder remained horizontal, independent of the position of the tunnel which was changed in accordance with the changing position of the sun (see fig. 2). The experiments were carried out with *a.* the feeder attached to the separately mounted black background without test tunnel; *b.* the feeder attached to the white background, mounted with the tunnel with white inner walls, and *c.* the feeder attached to the black background, mounted with the tunnel with black inner walls. During the experiments of series *a.* the position of the background was corrected as if mounted with the test tunnel.

Every experiment started with the collection of some foragers, being about to start from the alighting board of their hive, on a piece of filter paper impregnated with sugar syrup. These bees were introduced to the feeder which was located at about 10 m distance from their hive. When the feeder was used inside the complete test tunnel, the bees were introduced to the feeder through the central opening in the back-

ground. After this the opening was closed with a white or black cork. This procedure was continued until 10 bees had been seen feeding from the feeder for some time. The departure flight of these bees was observed, when necessary through the orifice of the tunnel as described before (p. 47), and after that observation of the feeding place was continued during one hour, looking forward to the returning of these bees or to the arrival of recruits. The bees originally introduced to the feeder could not be discriminated from the possible recruits. Every experiment was repeated four times.

The experiments of series 2 *a* gave the results which were to be expected. After having fed for some time a bee performed the well-known orientation flight around the feeding place. The bee hovered around the feeding place, first close to it and with the head directed towards the feeder, then in wider and wider curves for some seconds before the bee ultimately returned to the hive. Soon more and more recruits went to the feeding place, and the feeder had to be supplied with syrup a couple of times in order to meet the transport capacity of the increasing stream of foragers.

Quite different were the results of the experiments of series 2 *c* in which the feeder was mounted inside the black tunnel. When the bees introduced to the feeder had finished feeding, they flew up. But instead of performing the normal orientation flight as observed when bees are leaving a feeding place, they immediately exhibited an abnormal behaviour. The orientation flight was almost absent and most bees had left the tunnel within two or three seconds. Sometimes the observer got the impression that a bee when turning towards the feeder, tried in vain to perform some orientating movements. But in the next moment such a bee was swept outside. Other bees were greatly disorientated, spinning around and dashing against the walls as described above (p. 49). About half of the bees performed some kind of orientation flight after passing through the orifice of the tunnel and before returning to the hive. The ultimate result was that during the observational period of one hour no single bee returned to the feeder in either of the four experiments of series 2 *c*. Some bees were observed flying around the orifice of the tunnel, but not one succeeded in entering the tunnel. It could not be established whether these were returning bees or recruits. Needless to say that in these experiments the abnormal behaviour of the bees in the black tunnel can not be considered as a positive phototactic flight reaction, due to disturbance or such like.

The experiments of series 2 *b* carried out in the white tunnel gave rather irregular results. The departure flight of the bees introduced to the feeder showed great individual differences. Some bees performed rather normal orientation curves with the head directed to the feeder, but other bees flew out of the tunnel almost immediately

after having finished feeding. Eleven bees out of the four groups of ten that were introduced to the feeder were rather highly desorientated as was obvious from the capricious curves described during departure, while 8 out of these eleven bees were seen falling one or more times to the floor before they passed through the orifice. Again a number of bees performed orientation flights outside the tunnel. The over-all result of these four experiments was that in only one experiment the training to the feeder was a complete success with a great number of returners or recruits foraging on the feeder. Another experiment of this group ended in a complete failure. In the first half hour three bees were seen trying to enter the tunnel, but only one bee reached the feeder and actually fed on the syrup. During the two other experiments a fairly regular stream of bees was visiting the feeder, though not more than about 10 bees were seen at a time upon the feeder. It was observed that many bees had some difficulties in entering the orifice of the white tunnel. Such bees were seen hovering around the orifice during several seconds, and when entering the tunnel they sometimes again returned before having advanced half-way. Then hovering around the orifice was resumed, and in this way it could take a considerable time for a bee to reach the feeder. It was observed more than once that a bee which after some efforts had entered the tunnel through the orifice, suddenly dashed against a wall or against the floor. Then some distance was covered walking at high speed to the orifice, after which the bee flew outside through the orifice and was lost sight of. Other bees, however, were seen to enter the orifice almost immediately after arrival, and then the feeder was reached within a few seconds. The observer got the impression that the individual capacities of the bees to leave or to enter the white tunnel were rather divergent.

In the experiments of series 2 *a*, *b*, and *c* there was one factor which was difficult to control. We must assume that the feeder with sugar syrup had to compete with other available crops. Although most of the experiments were carried out in a period without important crops, the interest of the bees in returning to the feeder could not be considered as a constant factor on different experimental days. Therefore, a third series of experiments was carried out, in which the tendency of the bees to return to a certain point could be assumed to be continuously strong.

3. *Release of bees within the test tunnel, placed in strange territory*

In these experiments foragers obtained from hives at a distance of more than 10 km from the laboratory were liberated in groups of 50. It is well known that bees transported over such a distance do not return to their hive, and that a re-orientation at the place of liberation occurs. Bethe (1898) was the first who carried out such experiments, in which bees were liberated from a box in strange territory.

After collecting, our experimental bees were kept in the laboratory in cages placed in an incubator at 30° C and supplied with food and water. The bees were used within a week, as soon as weather conditions permitted. For each experiment 50 bees were introduced into a box, 15 cm long, 15 cm high, and 10 cm wide, and made of hardboard. The box with the bees remained in the incubator for half an hour. Then the box was attached outside the tunnel at the centre of the background. An

opening 3 by 1 cm connected the box with the tunnel (see fig. 2). This opening was closed by a strip of plaster. This strip could be pulled away through the orifice of the tunnel with the help of a thread fastened to the plaster. At this moment the bees were liberated and the experiment started. These experiments, too, were carried out under three conditions, namely with *a*. the box attached to the separately mounted black background without test tunnel; *b*. the box attached to the white background mounted with the white tunnel, and *c*. the box attached to the black background mounted with the black tunnel. During the experiments of series 3 *a* the position of the background was corrected as if mounted with the test tunnel.

TABLE I

Box attached to black background mounted without tunnel

Series 3 *a* Exp. No.	Number of bees introduced to the box	Number of bees returned to the box during the second half hour of the experiment	Number of bees found in the box after one hour
1	50	51[1]	0
2	50	48	1
3	50	45	3
4	50	47	3
	200	191	7

[1] The number 51 may be the result either of wrong counting or of a bee from a neighbouring hive.

In the experiments of series 3 *a* the behaviour of the departing and returning bees was observed during the first half hour after liberation. The departing bees showed the well known orientation flight, at first hovering around the opening of the box with the head directed towards it, and then flying around in ever increasing curves. Some bees returned soon, while others disappeared out of sight. When returning, the bees obviously had no difficulty in refinding the opening in the background.

TABLE II

Box attached to black background mounted with black tunnel

Series 3 *c* Exp. No.	Number of bees introduced to the box	Number of bees returned to the box during one hour	Number of bees found in the box after one hour
1	50	3	5
2	50	1	4
3	50	5	7
4	50	3	4
	200	12	20

During the second half hour the returning bees were collected at the "entrance" of the box with the aid of the catching-tube. After that the number of bees in the box was counted. The respective numbers are

presented in table I, p. 52. This table shows that in all four experiments the bees were very successful in reorientating to the new position of their "hive". This is a great contrast to the results of the four experiments of series *c* in the black tunnel which are presented in table II, p. 52.

In this series of experiments the recording procedure was somewhat changed for several reasons. First, it was impossible to collect the returned bees at the entrance of the box and to observe them at the same time through the orifice of the tunnel. Moreover, the number of bees which returned to the box was so small that it could be established by counting the bees that were observed to reach the entrance of the box via the orifice of the tunnel. While doing so, the same bee may have been counted more than once when returning after another departure, but as will be seen from table II this can not have influenced the results materially. The behaviour of the departing and returning bees was considered to provide more information about their ability or inability to orientate in the tunnel than the mere fact that only some bees succeeded in returning to the box. Therefore, the behaviour of the departing and returning bees was observed, while, at the same time, the number of bees returning through the orifice of the tunnel in an attempt to reach the entrance of the box was counted during one hour after liberation.

Table II illustrates that only very few bees succeeded in returning to the box. Some peculiar features of the behaviour of the bees in the black tunnel will be exemplified with the aid of experiment No. 1 of this series.

At 14.30 the bees were liberated. The angle of incidence of the rays of the sun was about 35 degrees and the tunnel was mounted accordingly. The bees departed one after another at intervals of some seconds and after a few minutes at larger intervals. True orientation flights around the entrance of the box were almost absent. Many bees were out of the tunnel within two or three seconds. Sometimes the head of a bee was directed towards the entrance of the box for some moments, the dorsal side being directed towards the sun. But in some irregular curves such a bee was out of the tunnel before a true orientating flight was performed. Other bees struck a wall, or they fell on the floor. Then they ran towards the orifice for some distance, started flying again and were swept out of the tunnel. At 14.45 some thirty bees had left the tunnel, and a number of them, approximately twenty, was flying around the tunnel. When approaching the orifice of the tunnel from outside, the bees described a very characteristic trajectory. The observer got the impression that the orifice of the tunnel was closed by a concave, transparent membrane, at the surface of which the bees were gliding off whenever they tried to enter the orifice. At 14.46 one bee entered the tunnel. In a sequence of flying towards the background, spinning around, falling on the floor, walking towards the orifice for some distance, starting again, etc. the efforts continued for about five minutes. Then the bee flew out of the tunnel. At 15.01 a bee entered the tunnel and flew somewhat zigzagging towards the background of the tunnel. The animal alighted about 10 cm above the entrance of the box and remained there for 50 seconds. Then she started again, alighted at the entrance and disappeared in the box. At 15.04 a second bee reached the entrance, this time without intermediate landings, but after several attempts to penetrate into the tunnel for some distance. At 15.19 again a bee flew towards the entrance of the box. At 15.30 the experiment was finished. A number of bees, approximately ten, was still trying to enter the orifice of the tunnel. Flying from different directions

towards the orifice, their course was time and again diverted by the "invisible membrane" so as to make them miss the orifice. There were found 5 bees in the box of which at least two had not left the box as follows from the fact that three times during the experiment a bee had been seen entering the box.

During the experiments of series 3 *b* with the box attached to the white tunnel, the bees were rather more successful in returning to the box. The results of the experiments of series 3 *b* are presented in table III (p. 55).

Just as in the conditioning experiments in the white tunnel described above (p. 50-51), it was observed that the behaviour of both the departing and the returning bees was rather divergent. Several departing bees performed an almost normal orientation flight around the entrance of the box. Other bees, however, performed spinning movements, sometimes falling to the floor of the tunnel. As for the returning bees, some of them were seen to enter the orifice of the tunnel without hesitation, while others performed curves reminiscent of those made by bees trying to enter the orifice of the black tunnel as described above (p. 53). Once they had entered the tunnel, the behaviour of the bees was still greatly divergent. Some bees were observed flying quietly towards the entrance of the box, while others showed several signs of disorientation as described for the bees returning into the black tunnel. Judging from the regularity with which bees were seen departing from or returning into the tunnel, orientation was much improved as compared with the experiment in the black tunnel.

Three further experiments were carried out on the reorientation of bees released in strange territory.

In experiment 3 *d* a swarm of bees with a caged queen was introduced into a small hive containing three frames. This hive was closed and mounted to the black tunnel as described above for the small box. The experiment was favoured by excellent weather conditions (no clouds, air temperature 26° C). After liberation of the bees, the tunnel was observed during one hour. The bees appeared to be highly disorientated. Their behaviour was as described above (see p. 48–49) for bees in the black tunnel. During the observational time about 200 bees were seen to fly out of the entrance. Only three bees out of the cloud circling around the orifice of the tunnel succeeded in penetrating the tunnel up to the background where they alighted. None of them reached the entrance as they were swept out of the tunnel in an attempt to start in search for the entrance.

Bethe (1898) observed that bees whose antennae had been amputated returned to their displaced hive, whereas the normal bees collected at the old position. Wolf (1926, 1927) surmised that the curves performed during the orientation flight would be registered by the antennae. Kalmus (1937) and Kuwabara (1952) assumed that the antennae are involved in some sense of rotation. However that may be, it is clear that in our experiments this supposed orientation ability did not counterbalance the photic disorientation in the black test tunnel.

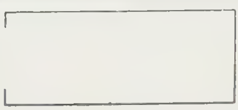
The objection may be made that the disorientation of the bees in the above experiments was in part due to the restricted space available to

the bees in the tunnel. Therefore, some further experiments were carried out with bees released in strange territory in order to investigate whether they can orientate in a restricted space when the illumination conditions meet the necessary requirements.

Groups of 50 "foreign" bees were liberated from a box attached as described above (p. 51–52) to tunnels of a somewhat changed design. In experiment 3*e* use was made of a tunnel of about the same dimensions as the tunnel used in the experiments of series 3 *b*, *c* and *d*. But instead of being rectangular, the tunnel had the shape of a truncated pyramid. The length of this tunnel was 300 cm, the background measured 90 × 90 cm, while the orifice directed towards the sun measured 120 × 120 cm. When this tunnel was directed towards the sun, the background was not the only illuminated part of the inner surface, as now the side walls, ceiling and floor were directly illuminated as well. The inner walls were painted dull white. The tunnel used in the experiments of series 3 *f* had the shape of a truncated cone and was much smaller, the dimensions being about one third of those of the tunnel used in series 3*e*, namely: 100 cm in length, while the circular background was 30 cm in diameter, and the circular orifice was 45 cm in diameter. Here, too, all

TABLE III

Box attached to white background mounted with white tunnels of different design

	Type of tunnel	Number of bees introduced to the box	Number of bees returned to the box during the second half hour of the experiment	Number of bees found in the box after one hour
Series 3 *b*				
Exp. No.				
1		50	14	1
2		50	29	6
3		50	9	0
4		50	17	5
		200	69	12
Series 3 *e*				
Exp. No.				
1		50	39	4
2		50	44	2
		100	83	6
Series 3 *f*				
Exp. No.				
1		50	48[1]	–[1]
2		50	41	6
		100	89	6

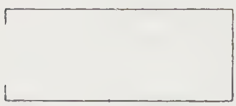
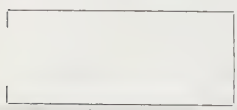
[1] This experiment was interrupted after 25 minutes by a sudden shower (see p. 56).

the walls were painted dull white and directly illuminated by the sun when the tunnel was directed towards it. The results of the experiments of series 3 *e* and 3 *f* are presented in table III on p. 55. They are almost identical to those of the experiments with the box attached to the black background without tunnel (series 3 *a*, see table I on p. 52). Even in the small tunnel it could easily be observed that departing bees had no difficulty in making small scale orientation flights, and their returning, too, was unhampered. One experiment with the small tunnel in particular illustrates the normal orientating behaviour of the bees. About 25 min after liberation of the bees a sudden shower accompanied by a sharp fall in temperature drove the bees back into the box within a few moments. Ten minutes afterwards no returning bees were seen. In the box 48 bees were found, 5 of which were dead. Because of the unambiguous results of the experiments of series 3 *e* and 3 *f*, each experiment was carried out only two times.

Obviously, the less directed character of the illumination in the two tunnels, used in series *e* and *f* respectively, enabled the experimental bees to make use of their normal photic orientation faculties in returning to the opening from which they had been liberated. In other words, the ability of a bee to perform normal orientation movements appears to depend upon the ratio of the intensity of the light stimuli received directly from the source (the sun) to the intensity of the light stimuli received indirectly by scattering and reflection.

TABLE IV

Drift towards the light source (the sun) and orientation ability of bees (ability to fly, to perform orientation flights, to return to a feeder, or to return to the point of liberation) under different illumination conditions; results of the experiments of series 1, 2 and 3, described in section IV A

Type of tunnel	Inner surface	Ratio illumination intensity side wall: background	Orientation ability	Drift towards the source (the sun)
no tunnel	—	$\pm$ 0.1	normal	none
	white (completely illuminated)	0.2–0.1	normal	none
	white (partly shadowed)	0.05–0.02	moderate, with extreme individual variations	moderate
	black (partly shadowed)	10^{-4}–10^{-5}	almost absent	strong

Although the available data do not enable us to make an exact comparison of the degree of disorientation in the respective experiments of series 1, 2 and 3, the results of these experiments are tentatively tabulated on p. 56. This table (no. IV) was devised to illustrate the relation between the illumination conditions and the ability of bees to use their photic orientation faculties as observed during these experiments. The illumination conditions in the experimental space were characterized in a fairly suitable way by the ratio between the illumination intensity of a vertical plane including the sun (a side wall of the tunnel for instance), and the illumination intensity of a plane irradiated perpendicularly by the sun (the background of the tunnel for instance). (The measuring technique is described in section V, p. 68.) These two illumination intensities were chosen because the former gives a suitable measure for the average illumination intensity accomplished by scattered light (the sky) and by light reflected from the environment, while the latter, achieved by perpendicular irradiation from the light source (the sun), is the highest illumination intensity to be found at the measuring spot.

A more unambiguous estimate of the illumination conditions at the experimental spot could, of course, have been obtained from measurements in more than two directions. Up to the present, however, the usual procedure during experiments on photo orientation has been to measure "the" illumination intensity in the experimental space, appreciating neither which light intensity was actually measured, nor which might be the importance of the measured light intensity in bringing about the observed phototactic reactions in the experimental animals. To illustrate this it might be imagined that in the experiments with bees described above only one illumination intensity had been measured as being representative of "the" experimental illumination intensity, namely the illumination intensity of the background Under the different experimental conditions described above -isolated background, and background with black or white tunnel of different design—"the" illumination intensity would have been found to have very much the same value namely that of full sunlight. In view of the highly divergent behaviour of the bees under the respective illumination conditions this would after all not have been a very illuminating result.

With the purpose of measuring the total influx of light into a plant community, Wassink and van der Scheer (1951) measured the illumination intensity in six mutually perpendicular directions in full sunshine on a lawn, in the shadow between or under trees, etc. The ratio between the lowest and the highest of these 6 illumination intensities was generally about 0.1, and only seldom reached the value 0.03, for instance when the measuring spot was situated between trees. Obviously these values

of the ratio under consideration are characteristic for the normal illumination of various environments which can be regarded as the natural habitat of insects like bees.

It will be seen from table IV (p. 56) that the more the ratio between the two selected illumination intensities exceeds values that are encountered in the normal illumination of the animal's habitat, the more the photic orientation of bees is deranged and transformed in a drift towards the light source.

It must be emphasized that the ratios mentioned above are presented only to give a rough idea of the order of magnitude of the illumination factors involved in the reactions in question. The exact values of these ratios are determined by the total structure of the environment. For a thorough discussion of these aspects of environmental illumination and their consequences in photo orientation, the author refers to the discussion (section V).

4. *Experiments with walking bees*

In order to give a more illustrative image of the drift towards a light source some records of paths of wingless bees walking under laboratory illumination conditions are presented in plate I. This plate shows that wingless bees can be forced into conditions incompatible with survival by a lamp in a dark room. The paths of ten bees were traced on a plate of fire-proof board, which was covered with aluminium paint and after that sooted. A 500 watt overrun incandescent lamp mounted in a hole in the plate was the trapping light source and at the same time a source of lethal heat. Five bees were put in the left-hand white circle and five in the right-hand one, both at a distance of 30 cm from the lamp. Within a few seconds the bees walked towards the lamp where they were scorched. Similar results were obtained when intact flying bees were liberated in the vicinity of the lamp.

B. EXPERIMENTS WITH FISHES AND OTHER AQUATIC ANIMALS

The experiments with aquatic animals, and those with birds to be described in this section were carried out in order to ascertain whether the results previously obtained with insects could be reproduced with representatives of widely separated species of animals. With the data presented in section III B and C in mind, this result may be expected from *a priori* reasoning.

1. *Observations during fishing with light*

Before dealing with the laboratory experiments a description will be given of the movements of a number of aquatic animals as observed

Plate 1. The paths and the scorched cadavers of ten wingless bees liberated in a dark room in the vicinity of a 500 watt overrun incandescent lamp.

during fishing with light. These observations were made during the nightly capture of sardines, anchovies and other fish with the help of light sources in the Mediterranean near the island of Ischia (close to Naples, Italy) and in the bay of Banyuls (south coast of France close to the Pyrenees). The fishermen used two petrolgas lamps (capacity about 6000 candles each) mounted about 0.5 m above the surface at a mutual distance of about 1.50 m. When considering the movements of fishes or squids at some distance both lamps could be considered as one light source. But small animals like *Heteronereis* showed a distinct concentration under each lamp amidst the whole mass of animals gathered around both lamps. Some observations were made about the behaviour of animals in the light field around a small electric underwater lamp (6 volt, 3 amps.) constructed by the author.

The behaviour of the following animals is described below:

a. the "*Heteronereis*" form of a marine worm (*Platynereis Dumerilii*), *b.* the cephalopod *Loligo vulgaris*, and *c.* some fishes.

a. The swarming of the *Heteronereis*[1] forms has been described more than once. The breeding habits exhibit a lunar periodicity which seems to be characteristic for each species (cf. HEMPELMANN 1928–34, KORRINGA 1957). When swarming the animals appear swimming near the surface during the night and they are readily attracted by artificial sources of light (LILLIE and JUST 1913; FAGE and LEGENDRE 1923a, 1923b; RANZI 1931).

The concentration of *Platynereis Dumerilii* around a lamp usually began with the appearance of a few males. When the larger females began to appear they were each soon surrounded by several males, which swam rapidly in narrow circles about the female (the "dances"). Numerous males which were not engaged in circling around a female were strongly attracted towards the lamps. Some efforts were made to analyse the paths of these animals in the vicinity of the lamps. After observation of the phenomenon during some nights the general impression was that the paths were generally concave towards the lamps, and that an individual worm swam along capricious loops with a common junction under a lamp. The diameter of these loops varied between some

[1] The name "*Heteronereis*" refers to the pelagic fully mature males and females of a number of marine worms. Because of the differentiation of the anterior atoke and the posterior epitoke part of the body during maturation, the animals have formerly been looked upon as a separate species during this phase of their life. The posterior segments bear the gametes and their parapodia are adapted to the swimming habit. In comparison with the sedentary condition of the worms, the "*Heteronereis*" has enlarged sense organs (eyes, antennae). The classification of these worms is complicated; HAUENSCHILD (1951) demonstrated that the mature nereid form and the mature heteronereid form of *Platynereis Dumerilii* are not different forms of the same species (dissogony), but that they are separated species.

decimeters and some metres. As soon as the lamp moved relative to the water the parts of the paths directed from the lamp were shortened and the parts towards the lamp lengthened. With increasing speed of the lamp relative to the water the worms followed in gradually more stretched paths until they followed the lamp in mass along more or less undulating paths. There was no longer time for random movements. Similar paths were observed in small shrimps (Mysidae etc.).

b. Loligo vulgaris appeared rather regularly around the lamps. Once a specimen of about 10 cm in length could be seen moving around the lamps for half an hour along more or less capricious, undulatory paths. The paths were not concentric around the lamps since the animal passed regularly directly underneath the lamps after having performed curves with a radius up to about 10 m around the lamps. Obviously the paths were for the greater part concave towards the lamps, or put in other words, the chance that a curve was made directed from the lamps was much smaller than for a curve towards the lamps. Similar paths were observed in larger *Loligos*. Sometimes the *Loligos* swam in a schooling formation.

c. Belone belone, which is a typical surface swimmer, could be observed fairly exactly. The paths of specimens of about 50 cm in length were similar to those of *Loligo vulgaris*. But sometimes the regular swimming was suddenly interrupted by peculiar and uncoordinated dashes out of the water or along the surface which sometimes ended under a lamp. There some fishes remained swimming parallel to each other for a considerable time thus compensating the relative movement between lamp and water (the boat lying at anchor in the current). It was observed more than once that a *Heteronereis* was captured by these fishes.

The appearance of *Clupea pilchardus* and *Engraulis encrasicholus* under the lamps was often preceded by the appearance of rising airbubbles originating from compensation of the increasing volume of the gas bladder in the ascending fishes (cf. p. 36). Sometimes the sardines and anchovies remained so deep that they could hardly be seen in the very clear water at the spot. But on other occasions they could be observed very distinctly at depths of some meters. Sometimes the fishes were swimming in some schooling formation, appearing under the lamps in waves. Other times they were swimming criss cross and at high speed making sudden turns, tilting movements, or leaps out of the water.

It has been observed in aquaria (VERHEIJEN 1953) that the parallel schooling formation of herrings is abandoned temporarily when the animals are feeding, and that sudden turns and upward movements are performed when small prey animals are pursued. In the meanwhile similar movements were observed in sprats, sardines and anchovies living in aquaria. Under the fishing lamps it could be observed some-

times that a sardine captured a *Heteronereis*, but in all probability the greater part of the strange movements was not due to hunting for prey but to uncoordinated or disorientated movements resulting in an agitated swimming hither and tither.

The fishes disappeared out of sight when noise was made on board of the boat, or when predators like *Loligo* appeared on the scene. After these rivals had been captured with a spear the fish soon reappeared under the lamps. Evidently they can withdraw from the attraction of the lamps when disturbed by acute danger (cf. p. 23). Normal behaviour patterns, like the "dances" of the *Heteronereis*, and schooling and feeding of fish, were regularly observed around the lamps. The observed behaviour is obviously a mixture of the drift towards the lamps and the performance of normal activities.

Some observations were made around a small electric underwater lamp (6 volt, 3 amps.) at the same localities. There was no essential difference in the behaviour of several marine organisms in the vicinity of this small lamp as compared with the behaviour observed under the strong petrolgas lamps of the fishermen. As during some of these observations the boat was floating without anchor on a sea almost as smooth as a mirror, two species of fishes could be observed quite exactly: small *Belone belone* (about 20 cm in length), and *Atherina* sp. Two reaction patterns were predominant. 1. Both species were observed standing almost motionless in the water for some minutes, thereby sometimes touching the lamp with their head. 2. Then suddenly some flashing movements were made during which peculiar tilting and turning was conspicuous. Thereafter the fish again became almost motionless, sometimes approaching the lamp very slowly. Both species could be driven off by making noise in the boat or in the water.

2. *Laboratory experiments*

In the laboratory attraction experiments were carried out with some cyprinoids, with the three-spined stickle back (*Gasterosteus aculeatus*), with small pikes (*Esox lucius*)[1], and with the marine species *Gobius minutus* and *Belone belone*. At first completely negative results were obtained. In rectangular tanks with black walls, the largest of which was 230 by 150 cm and 60 cm deep the fishes were not disturbed by the above mentioned small underwater lamp. No abnormal swimming movements were observed. A concentration around the lamp was not observed either, but might perhaps have been demonstrated statistically by counting at regular intervals the number of fishes being present in certain regions of an experimental tank. But it was considered to be more

[1] These fishes ware kindly supplied bij Mr. K. Willemsen, Rijksinstituut voor Visserijonderzoek, IJmuiden.

demonstrative when illumination conditions could be devised which would evoke distinct "tropisms" of the fish towards the light source.

Wondering why fishes in a tank were not attracted by a small lamp while the same lamp was highly attractive in the Mediterranean water, we supposed that the only reason could be the difference between the illumination intensity of the environment. When the lamp was immersed up to one metre in the clear water of the Mediterranean the only thing which could be perceived was the glaring light source surrounded by a weak bluish haze due to scattered light. This point source of light was still quite sharply visible when dropped down to a depth of 15 m. When the same lamp was immersed in a tank, the whole tank was fairly brightly illuminated, and could be taken in at a glance, even when the largest tank (230 by 150 cm and 60 cm deep) with black walls was used. It was supposed that the illumination of the walls of the tank was the factor which prevented the "positive phototropism" of the experimental fishes.

When a given lamp is used in a given tank the illumination intensity of the walls can only be reduced by applying a hood with a diaphragm. It must be emphasized that a diaphragm with not too small an aperture does not change the field of light within the beam as far as it is determined by the light source. But it does change this light field when the light reflected from the environment is taken into account. The smaller the diaphragm the weaker the environment is illuminated and the weaker the intensity of the reflected light that enters the beam. In other words the light field in the beam becomes the more unidirectional the smaller the beam.

On the basis of these considerations some further experiments with marine species were carried out in a large aquarium the dimensions of which were 240 by 100 cm and 85 cm deep. Clear sea water was kept running through this tank at all times. The light source was placed outside the tank. The light from this source could penetrate into the aquarium through a glass side 100 by 85 cm. The opposite wall was fitted with a screen of blackened waterproof hardboard. The diameter of the beam could be regulated by drawing out the hood, thus changing the distance between the diaphragm and the lamp. The boundaries of the beam were made very sharp by two additional diaphragms mounted within the hood. These diaphragms intercepted the light reflected by the black inner surface of the hood.

When using a 6 volt 3 amps. lamp the whole tank could be taken in at a glance when the beam illuminated a circular area of the opposite black wall 80 cm in diameter. A fish swimming outside the beam could easily be detected. When the beam illuminated an area 10 cm in diameter the tank could no longer be surveyed and a fish outside the beam

could not be detected. When a stickle back (these fishes were tested both in sea water and in fresh water with identical results) happened to enter this beam, the fish immediately turned towards the light source and began to swim in that direction. When slightly horizontal as well as vertical deviations of the fish were compensated by turning the lamp it was nearly always possible to make the fish dash against the wall at the spot where the beam entered the tank. It is clear that these corrections of the beam direction are allowed, since the fish could not get out of the "beam" when the same lamp was suspended freely, without hood, under the sea level. The paths followed by the fish in their swimming towards the light source varied between almost rectilinear, making correction of the beam direction superfluous, and more or less undulating. Sometimes a fish deviated in one direction so that it swam gradually more and more transversely with respect to the beam. This could result in the fish leaving the beam, but also in ultimately turning into the beam direction. Sometimes the stickle backs reacted very violently upon entering the beam, darting over some distance towards the lamp. But then they were mostly out of the beam before its direction could be corrected.

The wider the beam, the less consistent the trapping effect appeared to be, and with a beam illuminating an area of the opposite wall 80 cm in diameter, the drift towards the lamp was only seldom evoked and it could hardly be recognized.

Comparable reactions were obtained with *Gobius minutus*. These fishes reacted most distinctly when they were caught in a narrow beam while swimming. But they are bad swimmers and continuously tend to sink. To compensate for this sinking they swim slantingly upwards, thus describing a kind of saw-tooth path, and therefore it was rather difficult to keep them in the beam. Nevertheless they could be led towards the lamp from distances of about 1 m. When the beam found them while resting at the bottom they could only rarely be lifted from the bottom. Here the strong illumination of the bottom might have been an interfering factor.

Very strange reactions were observed in *Belone belone*. When their tank was illuminated by a horizontal beam these otherwise quite tame fishes dashed through the tank hurting their long "beaks" against the walls. Although they were occasionally seen swimming at high speed through the beam, as did the stickle backs when reacting very strongly, the whole picture was rather more complicated. *Belone* are normally typical surface swimmers. But when the illumination intensity of the tank was somewhat lowered they suddenly sank to the bottom. This reaction has not yet been analysed further. But it might be assumed that this sensitivity to changes in light intensity complicated the behaviour in the beam.

Tentative experiments were made with some Cyprinidae, but these fishes were swimming so capriciously and fast that they had traversed the beam before a distinct reaction could have developed.

An ideal experimental animal was found in pikes of 5–10 cm long. Experiments were carried out in a plexiglass tank 80 by 40 cm and 20 cm deep. The bottom and the walls could be screened with plates of black waterproof hardboard. The reaction of the fishes could be well observed from above. Each experiment started with searching until the generally immobile pike was illuminated by the beam. The following chain of reactions was then observed: 1. turning of the eyes towards the light source, 2. turning of the body in the direction of the beam, and 3. swimming through the beam until the perspex wall was touched at the spot where the beam entered the tank.

Generally reaction 1. changed gradually into reaction 2. Sometimes 1. was not clearly observed. If the pike's path deviated it was corrected by turning the beam as in the experiments with the stickle backs and the gobies. When suddenly a second beam was directed to the pike from another direction while the first beam was extinguished, the pike corrected his swimming direction accordingly. Similar reactions were noted when the beam was directed to the pike via reflection at the surface. The pike then swam through the beam towards the surface, and there corrected the swimming direction towards the lamp.

In addition to this "tractable" behaviour another and less schematical reaction was noticed. While a pike was "standing" or swimming in the beam in the direction of the lamp the animal sometimes suddenly turned about 90 degrees to the left or the right. When the beam struck the pike at an angle of about 90 degrees, and the right eye was illuminated (after the pike had turned to the left) both eyes were turned strongly to the left. But the pike moved with strange and contradictory fin and body movements and in this transverse position in the direction of the lamp. This was interpreted as being demonstrative of a failing effort of the pike to withdraw from the attracting effect of the lamp. Sometimes the pike actually did dart away suddenly.

These results were obtained with light sources of greatly varying intensity. The weakest light source used was a 6 volt 0.05 amps. lamp, the strongest was a Leitz Prado projector with a 250 watt lamp. The objective of this apparatus was replaced by a hood with a diaphragm. Obviously intensity is a rather unimportant factor in the trapping effect of light. These results are discussed in section V.

C. EXPERIMENTS WITH BIRDS

These experiments were carried out in the test tunnel with black inner walls described on p. 46. The tunnel was located in a dark room and

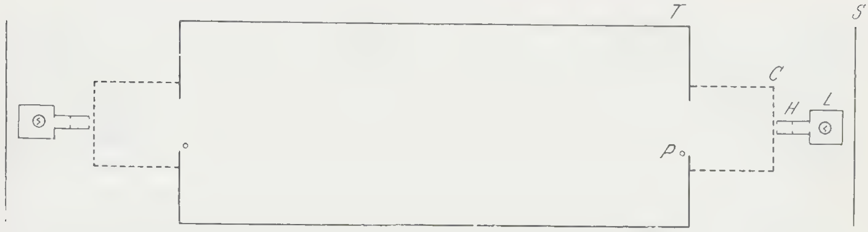


Fig. 4. Diagram of test tunnel used in the experiments with birds. T test tunnel; C cage; P perch; L light source; H hood with diaphragms; S screen.

used in a horizontal position. The arrangement is given in fig. 4. Both ends of the tunnel were closed with a hardboard plate in the centre of which there was an opening 30 cm in diameter. Each opening connected the tunnel with a cage of blackened wire gauze 50 by 50 by 50 cm. Behind each cage a light source with a hood and three diaphragms was placed. The lamps and the hoods were mounted in such a way that the two beams coincided exactly. The beams were prevented from illuminating the walls of the room by black screens placed behind each lamp. Across each tunnel opening a perch supported at both ends was mounted 10 cm below the centre of the opening.

When a bird was introduced in one of the cages the lamp at the other side of the tunnel was turned on. When the bird did not fly spontaneously towards this lamp, the animal was placed on the perch, and, by pushing away one support the bird was forced to fly. The experiments were carried out with five starlings (*Sturnus vulgaris*), two sparrows (*Passer domesticus*), and two little owls (*Athena noctua*).

The starling—a notorious victim of lighthouses (see section III C)—reacted very consistently. When the hood with the diaphragm was arranged in such a way that the whole opposite wall and parts of the four long sides were illuminated, the bird generally flew away in an arbitrary direction, and then the animal could be seen or heard walking around in the tunnel, pecking and flying against the walls, thus giving the impression of trying to find a way out. Under these conditions it could take several minutes before the bird flew into the cage behind which the lamp was burning. When we did not want to wait for this ultimate flying to this cage, the beam was made rather narrow, having a diameter of about 20 cm at a distance of about 3 m, and the bird was traced somewhere in the tunnel with this beam whereby the sound of the walking or pecking bird was used as a guide. Now a different behaviour was observed. The bird gazed upwards in the beam and walked in the direction of the light source, thereby looking more and more steeply upwards in the beam, the direction of which was corrected according to the position of the bird. Then suddenly the bird flew up and

moved through the beam into the cage. After re-establishment of the original positions of the two narrow beams the bird could be made to fly from one cage to the other and *vice versa* by switching on the lamps in turn. The bird generally flew against the outer wall of the cage where the lamp was burning. When the opposite lamp was switched on the bird either immediately flew back or first sat down on the perch, longing for the lamp at the opposite side, and after some intention movements the bird flew back to the opposite lamp. There were series in which a starling made a hundred flights between the two lamps within 5 minutes. With increasing illumination of the walls by a broader beam the bird gradually returned to the behaviour described above.

In very narrow beams steering was astonishingly precise. A beam widening from 0.5 cm at the diaphragm up to 5 cm at the opposite side of the tunnel was still followed. That the bird did not actually leave the beam could be checked in two ways. First when looking from behind the bird towards the opposite lamp in such a way that the bird screened off that lamp it could easily be observed that the lamp remained hidden behind the bird during the whole flight towards the lamp. Secondly the sharp outline of the beam obtained by two additional diaphragms mounted within the hood of the lamp would make the lamp invisible to the bird as soon as it would get out of the beam. This was checked by directing the beam of a lamp beside the bird sitting on the opposite perch. Next the beam was gradually turned in the direction of the bird: the animal did not fly away until being struck by the beam.

The same behaviour was observed when the same type of beam was used, whether produced by a weak lamp (6 volt, 0.05 amps.) or by stronger ones (see p. 64).

It was shown by some improvised experiments outside the tunnel that a starling can be made to fly along a beam over distances of at least ten metres.

With sparrows the results were different. These birds refused to fly from the perch when sitting in the beam, and when forced to do so they only seldom flew through the beam for some distance. They showed a strong tendency to go to sleep with the bill under a wing.

The behaviour of the little owls was between that of the starling and the sparrow. When sitting on the perch in the beam there was no tendency to gaze in the beam and the birds only seldom flew away spontaneously. But when forced to fly they fairly regularly flew towards the opposite lamp. However, as soon as the beam was made broader the owls tended to fly in other directions, thereby dashing against the walls.

In conclusion it seems to be proven that the typical flight of the starling towards the lamp resulted from the elimination of reflected light by the use of black walls and a beam of light. Like some fishes the

starlings showed hardly any tendency to fly or to walk towards a free lamp, even when the surrounding walls were black. Obviously the black walls reflected enough light to allow orientation in other directions. Under such conditions insects (bees) were strongly attracted by the light source.

V. DISCUSSION

The review of the literature and the results of the experiments described in the present paper indicate that the movements of widely separated species of animals like insects, fishes, and birds towards an artificial light source are "forced movements". It is shown that the animals can be forced into conditions which are incompatible with survival by certain changes in the illumination of their environment, brought about by the elimination of scattered and reflected light. Concepts such as "positive phototaxis", "intensity preferendum", "light optimum", etc., which are generally used in discussions on the movements of animals towards lamps, are of no use, because the movements towards an artificial light source have been proved to be the result of photic disorientation. Studies of conditions under which photic orientation is impaired are of interest in connection with the general principles of normal photic orientation. It is evident that the failing of the animal's orientation efforts under the illumination conditions outlined above, emphasizes the importance of the normal illumination in the animal's habitat. It is assumed that the animal's photic orientation mechanisms are adequately matched to this normal illumination.

A. THE ANGULAR LIGHT DISTRIBUTION

Some aspects of the concepts of "intensity" and "direction" as factors in the illumination of some experimental or natural light field were taken into consideration in section II. Up to the present the general procedure for characterizing a light field in studies of "phototactic" movements of animals has been to measure "the" light intensity at one or more points in the experimental light field or to determine "the" direction from which the light entered the points in question. It is, however, not an easy matter to determine "the" light intensity or "the" light direction at a given point, or to evaluate the importance of these factors in the evocation of the animal's photic reactions.

When studying the photochemical effect of light, for instance the photosynthesis of a plant, it is of importance to know the total influx of light impinging from all directions on the plant. In measuring this "total" illumination many difficulties have been encountered (cf. Wassink and van der Scheer 1950).

From the experiments described in the present paper it appears reasonably clear that this "total" illumination intensity can not be the only determining factor in photo orientation, and that certain directional features of the illumination are of crucial importance. It was suggested (p. 57) that an unambiguous estimation of the illumination conditions at a given spot can be obtained only when the illumination intensity is measured in several directions. For this purpose measurements were made under some illumination conditions with a photometer provided with a diffusion disk. The photometer could be rotated on a horizontal and a vertical axis. Readings were taken in directions mutually separated by 30 degrees. The illumination intensities measured at a given point in a number of directions are plotted on lines pointing from the measuring point in the corresponding directions. The 3-dimensional surface enveloping the end points of the "illumination vectors" forms the jacket of a "potato" of a more or less irregular shape. The shape of this "illumination-potato" characterizes the angular light distribution (in the following abbreviated as ALD) at the point in question in an unambiguous way.

It is of interest to consider which factors determine the shape of the "illumination potato" under different illumination conditions. It is easy to understand that it will have a protuberance at the part of its surface directed towards objects of a relatively high surface brightness (a light source or a bright object), and that it will have a dent at the part of its surface directed towards objects of a relatively low surface brightness (a dark object). The normal shape of the potato will be determined by the normal ALD which is effected by the following factors:

1. the nature of the light source;
2. the absorbing and scattering capacities of the media; and
3. the reflecting capacities of objects in the environment.

1. The sun and the moon are concentrated light sources of relatively high surface brightness. Their radiation enters the animal's habitat from a certain direction, casting deep shadows behind obstacles.

2. The media through which the radiation from the sun or the moon has to penetrate before it reaches the animal's habitat are the atmosphere and, in case of aquatic animals, the water. While passing through the media the composition and the direction of the rays of light are changed by absorption (all processes by which the light is transformed in other types of energy like heat or chemical energy), and scattering (all processes like deflection, refraction, and reflection by which the direction of the rays of light is changed). The result of the absorbing and scattering capacity of the atmosphere, the sky, can be considered as a light source with a hemispherical radiating surface presenting a

certain brightness pattern. The radiation from the sky has an undirectional character.

3. The reflecting capacities of the objects, which together form the entourage of the animal's habitat, are determined by the colour and structure of the surfaces of these objects.

The high contrasts between the illumination intensities of the lighted sides and of the shadow sides of an object produced directly by the parallel beam of the sun or the moon, are strongly toned down by the illumination of the shadow sides of the object by scattered and reflected light. This toning down is reflected by the shape of the "illumination potato".

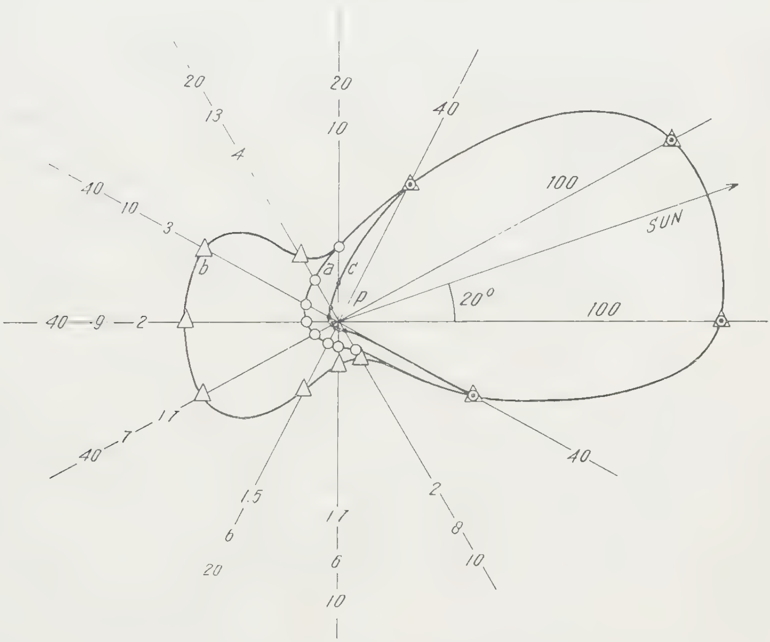


Fig. 5. Light vector diagrams. For explanation see text.

Fig. 5 shows the section of the "illumination potato" in a point p (about 60 cm above an exposed lawn) by the vertical plane including the point p and the sun. The light vector diagram a is the result of measurements at the point p under normal illumination conditions (sun altitude 20°). The diagrams b and c show the effect of a white and a black screen respectively. These screens measured 120 by 120 cm, and were placed vertically at a distance of 60 cm from the measuring point in a direction away from the sun. The light vector pointing towards the sun (the illumination intensity of a plane through point p irradiated perpendicularly by the sun) was put equal to 100. It will be seen from

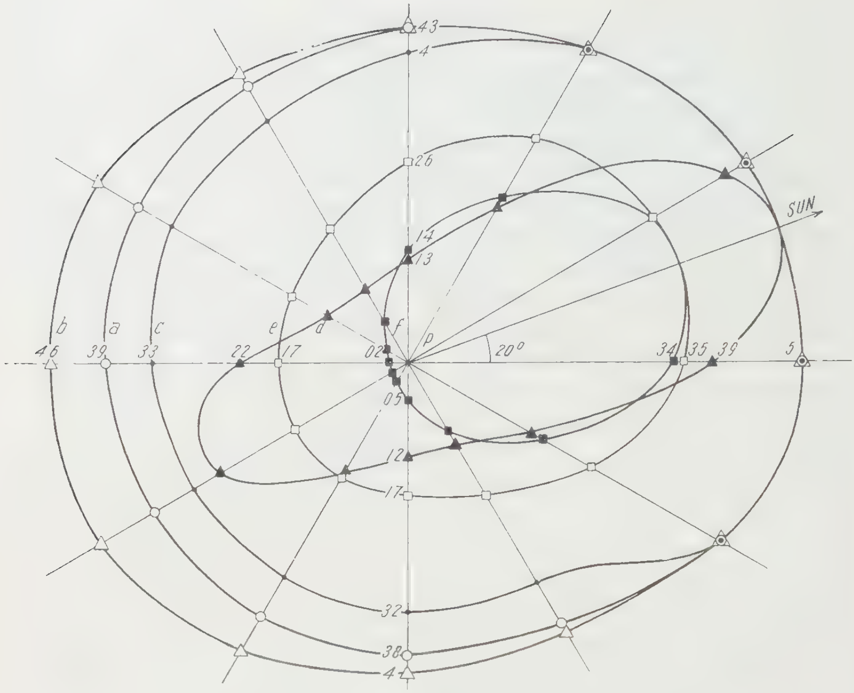


Fig. 6. Light vector diagrams. For explanation see text.

the values of the other light vectors that the ratio between the lowest and the highest illumination intensity ranges from 10^{-1} in case of the white screen to 1.5×10^{-2} in case of the black screen.

In fig. 6 the results of the same measurements are plotted logarithmically in the diagrams a, b and c. In these measurements the illumination intensity of the plane irradiated perpendicularly by the sun was put equal to 100.000 in an arbitrary unit of illumination intensity. This unit corresponds more or less to the lux. In view of the divergent visibility curves of different species of animals, and the corresponding relative importance of the absolute values of the illumination intensities, it was thought justified to use an arbitrary unit for plotting the results of these rather rough measurements. In diagram d are given the results of similar measurements, also plotted logarithmically, when the point p was located in the centre of the black test tunnel directed towards the sun as described in section IV, p. 46–47 (fig. 2). In fig. 6 the plotting is logarithmical because of the high difference between some illumination vectors within the black test tunnel as a result of the elimination of scattered and reflected light. In a natural terrestrial environment (a lawn, between trees, etc.) the ratio between the lowest and the highest

value of the light vectors ranges from 10^{-1} to 10^{-2} (cf. WASSINK and VAN DER SCHEER 1950), whereas this ratio reaches a value of 10^{-5} in the black test tunnel.

Also around an artificial light source abnormal values of this ratio were found. These measurements were made in a point p in the centre of a room (5 by 5 m and 3 m high) with light coloured (diagram e), or black painted walls and ceiling (diagram f). The floor was medium dark in both cases. The light source was a 500 watt overrun incandescent lamp, mounted at a distance of 1 m from the measuring point p at an angle of 20° above the horizontal plane. Even in the room with light coloured walls and ceiling the ratio between the lowest and the highest illumination intensity was found to be 10^{-2}. This was low enough to prevent bees from flying normally: they immediately dashed against the lamp. When similar measurements were made in a room with black walls (the usual situation in many experiments on photo orientation!) the ratio was found between 10^{-3} and 10^{-4}. Under such illumination conditions fishes and birds can still orientate fairly normally. They exhibit only a slight tendency to move towards a lamp (cf. section IV B and C).

A similar abnormal ALD is found in a room behind a window. This accounts for the results of the notorious experiments of LOEB (see p. 13) and the results of similar experiments of YERKES, V. BUTTEL-REEPEN, HOLMES and others described in section III A.

In a number of studies on submarine illumination attention has generally been focussed on the total illumination which is of importance for an estimation of organic production. The ALD has been studied only because it determines at any point the ratio between the total illumination and the vertical illumination. The latter can easily be measured with a photometer receiving light from the upper hemisphere only. Measurements of the angular distribution of submarine daylight has been made in different ways.

UTTERBACK and JORGENSEN (1936) used a photometer, the surface of which was first directed downwards and then turned upwards. The ratio of the upward to the downward illumination was found to be near 2%, varying with light colour and with depth. JOHNSON and LILJEQUIST (1938), PETTERSON (1938), ATKINS and POOLE (1940) and JOSEPH (1950) measured the angular distribution of subsurface light at different depths and in different ways. It has been found that just below the surface the "direction of maximum light" coincides fairly well with the direction of the refracted sun rays. This direction is called the "average obliquity" of the subsurface light. With increasing depth there is a distinct approach of the direction of maximum light towards the vertical: the average obliquity decreases with depth, the light as a whole becomes more vertical. The downward component of the submarine illumination always diminishes with depth. In the upward component, however, a minimum of intensity may be caused at a certain depth, since with further increasing depth the intensity of the upward component may rise as a result of reflection of light by the

sea bottom. WHITNEY (1941) presents experimental and theoretical evidence showing that, as depth increases, the ALD approaches a characteristic pattern which is symmetrical about a vertical axis.

The ratio between the upward and the downward component of the illumination at a given spot as measured by the above mentioned workers, ranges between 2 and 8%, depending on the interaction of factors like the solar altitude, clouds, depth, turbidity of the water, wave length of the measured light, and certain features of the measuring technique. It will be seen that under water the ratio between the lowest and the highest values of the light vectors falls within the range of such ratios under average terrestrial illumination conditions, namely 10^{-1}–10^{-2}. Obviously this range characterizes the normal ALD. Now it remains to be seen in which way the photic orientation mechanisms of animals are attuned to the normal ALD, and why certain changes in these conditions entail serious disorientation phenomena.

B. PHOTIC ORIENTATION MECHANISMS

Light is used by animals in controlling or steering their movements in space. From a teleological point of view these light-controlled movements or behaviour patterns are directed towards goals or are otherwise directive or purposive. The organization and coordination of receptors, nervous system, and effectors, which together form the "mechanisms" underlying these movements, must have developed during evolution as a result of the survival value of the resulting movements and behaviour patterns.

The controlling or steering of movements can be based upon:

1. The angular light distribution (ALD).
2. The presence of some particular source of light stimuli.

The movements steered with respect to the ALD are:

a. the adoption of a certain position (dorsal, ventral or other light reaction);

b. the movements towards a light intensity, which by various workers has been termed the "preferred" intensity, the light "optimum" etc.;

c. the movements towards a certain spot which is, at a given moment, of vital importance to the animal, for instance the water surface in case of an aquatic insect in need of ventilation.

The movements steered with respect to some particular source of light stimuli are:

a. the movements with respect to definite objects, such as parent, prey, sexual partner, nest, offspring, fellow member, etc.;

b. the movements with respect to heavenly bodies: the light compass reaction (a short review of recent work on this type of orientation was given by v. ST. PAUL 1958).

The following photic steering mechanisms enable the animal to perform the five types of movement mentioned above:

I. Mechanisms based on the comparison of the stimulus intensities acting upon the various eyes or other photosensitive surfaces ("diffuse light sensitivity").

II. Mechanisms based on the comparison of the stimulus intensities acting upon different regions of each eye, or in other words, the stimulus pattern in each eye (the term "stimulus pattern" does not refer to the detailed pattern of retinal images belonging to objects in the animal's environment).

III. Fixation mechanisms.

IV. The light compass mechanism.

The four types of steering mechanisms mentioned above have a dynamic character. The direction of the movement can be readjusted according to the needs of the moment.

The photic steering mechanisms I and II

There is a great number of papers describing the movements of animals (arthropods, fishes) after one eye has been covered or blackened: during walking or creeping the animal deviates towards one side (circus movements) while at rest the body is tilted on the longitudinal axis. When the animal has no contact with the bottom (during flying or swimming) it shows circling and sideward rolling. Recently SIMON (1954) observed similar phenomena in birds. It was these phenomena upon which LOEB based his tropism theory. According to this theory a normal bilaterally symmetric animal is forced to turn in a light field until the two photoreceptors are equally stimulated, and the circling and rolling after one eye is painted over would be the result of the impossibility to reach this condition (see p. 15). In his classification of orientating movements KÜHN (1919, 1929) termed the type of orientation based upon balance of (photic) stimulation (photo) "tropotaxis". Notwithstanding the many criticisms on LOEB's concept of "forced movements", the tropotaxis concept has retained a schematic and "forced" character throughout the literature.

It will be clear, however, that in a light field with a given ALD, the movements of an animal would be rather restricted when every inequality of stimulation of the eyes would evoke a reaction in order to restore the balance of stimulation.

In reality things are much more complicated. Tilting and circling were observed in insects not only after covering a whole eye, but also after covering parts of one or both eyes (AXENFELD 1899, HOLMES 1905, GARREY 1917, 1919, and others). Recently MITTELSTAEDT (1947, 1950) studied the photic and static equilibrial reactions in flying dragonflies. He found that the mechanisms of the dorsal light reaction are not

attuned to a point source of light but to large bright surfaces. In diffuse light of a given intensity each eye steers in only one direction as the insect with one blackened eye rotated around the longitudinal axis. But in a directional light field the normal dorsal light reaction was performed. It was found that at different light intensities different regions of each eye steered in different directions. MITTELSTAEDT concluded that the dorsal light reaction can not exclusively be explained by peripheral phenomena in the sense organs, and that the reflex idea about the dorsal light reaction is deficient for the elucidation of the observed orientating behaviour. Similar results were obtained by SCHOEN (1951) with fishes. In diffuse light of a given intensity each eye steered the rotation of the body around the longitudinal axis in only one direction ("einsinniger Lenker"). But in a directional lightfield each eye steered in both directions ("beidsinniger Lenker"). From a series of experiments with arthropods, SCHÖNE (1950, 1951, 1952, 1955, 1957) concluded that the function of the eyes and the statocysts is *not* to keep or to restore equilibrium in the sense of a *definite* spatial position of the animal characterized by a balance of stimulation of the eyes and the statocysts. In accordance with the re-afference principle (v. HOLST and MITTELSTAEDT 1950, v. HOLST 1954) these sense organs are used for controlling whether *any* position or direction of movement which is "*intended*" by the animal, is actually realized (cf. DIJKGRAAF 1953).

The way in which an animal uses the stimulus intensities acting upon the eyes and the stimulus pattern in each eye in controlling its movements may be described as follows: while an animal is moving about in its habitat the stimulus intensities acting upon each eye as a whole, and the stimulus pattern in each eye, change from one moment to another in a way which is characteristic of the ALD in this habitat. In the theoretical case of a completely homogeneous illumination no information can be derived from the eyes as to the position of the body. According to the re-afference principle the animal "knows" the photic stimulus patterns belonging to any position in its habitat. The animal "expects" these photic stimulus patterns while moving around, and makes use of them as a check on the successfulness with which it performs definite "intended" or "desired" movements. Slight discrepancies between "expected results" (efference-copy) and "checked results" (afference) are intercepted by correcting movements.

Recently such orientation mechanisms has been compared with a servomechanism. This is a machine in which an error in performance or output is fed back into the input in such a way that it opposes the signal responsible for the error. This is the principle of automatic control by negative feedback: the output is corrected and the error compensated. In the parlance of cybernetics the photic steering of a motion

such as the swimming of an aquatic insect towards the surface for ventilation may be described as follows: the photic stimulus pattern (the "desired" pattern) which is to be perceived after the "desired" upward position or direction of movement will be reached, is recorded somewhere and somehow in the central nervous system. At every moment of the ascent a definite photic stimulus pattern from the eyes is received in the central nervous system. The difference between the recorded "desired" pattern and the actually received pattern is the error signal. This error signal produces a change in the innervation of the relevant steering muscles, with the result that the animal corrects its direction of locomotion, thus again producing a change in the stimulus pattern. This, in turn, produces a change in the error signal, and this results in a further change in the innervation of the steering muscles until the difference between the "desired" pattern and the pattern actually perceived—the error signal—reaches a minimum.

As has been stated above the normal ALD at any point in the animal's habitat is characterized by a definite range and distribution of light vectors in this point (the "illumination potato"). During evolution the photic steering mechanisms of an animal must have developed in such a way that they reflect the normal ALD in the animal's habitat, for only on this basis is it conceivable that a future photic stimulus pattern which is to be perceived after the performance of a certain movement, is recorded in advance in the central nervous system as "efference-copy".

The close relation between the orientation of an animal into its normal position and the normal angular light distribution in its environment can be found again in other respects: the graded pigmentation of the body surface with concealing value known as "countershading" (a review of the relevant literature is given by COTT 1957); the relation between photic orientation and countershading in caterpillars (DE RUITER 1956); the distribution of bars in the retina of the eye of some fishes and its significance for the dorsal light reaction (BRAEMER 1957); etc.

When an animal moves about, abnormal ("unexpected") deviations from the "expected" sensory effects (efference copy), caused by experimental interference —for instance the painting of one eye or parts of an eye—lead to abnormal positions (tilting) and movements (circling) in arthropods, fishes and birds. Experimental evidence indicates that continuous afferent deviations from the efference-copy—as may be caused by diseases, injuries or experimental interference in receptors or effectors—are after some time adjusted in some way in the central nervous system so that normal positions and movements are restored as far as possible. The initial tilting and circling of a fish in which one eye

has been painted over, for instance, make way after some time for the normal position and locomotion.

Like artificially caused abnormalities in receptors and effectors, an abnormal ALD, too, provokes abnormal positions and movements. Dragon flies, for instance, can not fly in a dark room with a strongly illuminated white floor: they are disorientated by the steering mechanism of the dorsal light reaction as a result of which they crash down (Mittelstaedt 1950). Under similar experimental light conditions—which can be considered as an "inversed" ALD—disorientation has been observed in a number of other animals: the aquatic insect *Naucoris* dived to the illuminated bottom of its jar in an attempt to ventilate (Precht 1941), certain species of fishes swam upside down (v. Holst 1935, Braemer 1957). Abel (1954) observed fishes of non-identified species, swimming upside down in the blue cave at the island of Capri (Italy) at a spot where most of the light entered from beneath through an underwater opening in the rocky walls.

It will be clear from the above considerations and data that the abnormal ALD around an artificial light source (see p. 71 and fig. 6 diagrams e and f) can not fail to interfere with the photic steering mechanisms I and II as defined on p. 73. This must result in abnormal movements of the experimental animal.

The pattern of the normal ALD can be distorted up to various degrees of deformation. It seems convenient to discriminate between *a.* quantitative, and *b.* qualitative features of this deformation, though it must be admitted that they can not be separated, because the qualitative deformation—the abnormal shape of the "illumination potato"—is the direct result of quantitative changes in the values of the respective light vectors.

a. When the pattern of the normal ALD is "exaggerated" in such a way that the differences between some light vectors are strongly increased, a misleading of the animal by its photic steering mechanisms I and II must be the result, because the "desired" photic stimulus patterns are reached in a faulty position or direction of locomotion. A similar type of disorientation has been demonstrated in case of increased gravity force by Schöne (1957). When swimming from the surface towards the bottom the shrimps *Crangon vulgaris* and *Palaemonetes varians* took another direction when the gravity force reached the values 1.3 g or 2.2 g. Under these conditions the movements were steered in such a direction that again the checked stimulus pattern of the statocyst harmonized with the "desired" stimulus pattern.

The pattern of the ALD in the black test tunnel constitutes a fairly good example of this type of distortion of the ALD. The abnormal behaviour of bees in this test tunnel was described in section IV A. It is

clear (cf. p. 77ff) that steering mechanism III (p. 73), too, played an important role in causing the disorientation of the bees in the test tunnel.

b. The pattern can be distorted to such a degree that the light vectors which have normally the highest values, now become the smallest and *vice versa:* the whole pattern is reversed. On p. 76 some examples were given of animals disorientated under such illumination conditions. Animals moving above the lamp of a light trap are in a similar situation as far as the photic steering mechanisms I and II are involved in their photic orientation. This accounts partially for the crashing of insects into the trap.

III. *Fixation mechanisms*

The eyes of a great number of insects, fishes and birds contain an area with relatively highly developed visual acuity. This area is characterized by a smaller angle between two adjacent receptor units as compared with other parts of the sensitive surface.

For our purpose it is not very important whether there is a gradual increase in the angle between adjacent units towards the periphery of the eye (arthropods) or whether there is a circumscribed area centralis or even a true fovea with a very high density of receptor units (many fishes, birds and man). It is characteristic that the region with highly developed visual acuity is generally situated in such a way that the image of objects in front of the animal (a prey) can be made to fall on this region. In animals with laterally situated eyes this is obtained by the frontal situation of this region (insects), the temporal situation of this region (fishes, birds) or by extremely movable eyes (fishes, birds). In fishes and birds there are frequently two or more of these regions directed in different directions in each eye (for further details see PRINCE 1949, v. BUDDENBROCK 1952).

The ocular movements—or in animals with fixed eyes the movements of the head—steered by the fixation mechanisms are *a*. the movements to allow the image of an object which "interests" the organism to fall on this part of the eye, and *b*. the movements to keep the image there.

To account for the peculiar disorientation phenomena observed in the present study it will be necessary to analyse the interaction of the steering mechanisms determining *how* an object is fixed with the eyes, as well as the nervous mechanisms at "higher levels" of integration determining *why* a given object is fixed, and not another object.

Fixation mechanisms in insects have been studied by MAST (1923a, 1923b), DOLLEY and WIERDA (1929), CLARK (1928, 1931, 1933), LÜDTKE (1935), and BAUERS (1953b) (see p. 19), but no detailed information was obtained.

Therefore it will be necessary to acquaint ourselves with some results obtained in this field with vertebrates, including man, and particularly with penomena observed in patients with fixation failures and patients with certain mental disorders.

HESS and co-workers have studied the fixation movements in the cat and in fishes. HESS (1946) and HESS *et al.* (1946) observed typical movements of the eyes and the head after electrical stimulation of localized spots in the superior quadrigeminal tubercula and the pretectal region of the cat. It was suggested that these movements were reflex movements performed by the eyes, the head, and the body in order to carry the visual stimuli, perceived by the cat in the periphery of the visual field, towards the region of distinct vision. HESS termed these movements "the visual grasp reflex". By means of the Marchi technique definite relations were found between topographically defined parts of the tectal visual centres and certain peripheral regions of the retina. Similar experiments were carried out by AKERT (1947, 1949a, 1949b) with the trout (*Salmo irideus*). Electrical stimulation of localized parts in the tectum opticum of this fish induced coordinated movements of the eyes, the body and the fins, which would normally have been performed when a prey had been perceived somewhere in the periphery of the visual field. The results of a number of investigations in this field are given in tabular form by FAULKNER and HYDE (1958).

Obviously the movement patterns necessary for the capture of a prey are coordinated in the midbrain on the basis of incoming visual information. It is of interest to point to the fact that the visual perceptions evoked by the electrical stimulation of the tectum can only have had a neutral character (light-as-such), and not the character of what in the parlance of ethology is called a sign stimulus with releasing value (a prey).

The movements that keep the image on the fovea have recently been analysed in the human eye with the help of much refined techniques. It has been demonstrated that during fixation of a test point the human eyes are not stationary. Even during the most exact fixation the eyes are in constant motion. With the most sensitive recording techniques four types of these involuntary and unconscious eye movements have been found (ADLER and FLIEGELMAN 1934, RATLIFF and RIGGS 1950, DITCHBURN and GINSBORG 1953, DRISCHEL and LANGE 1956). The most conspicuous type is the rapid flick or saccadic movement by which the eyeball suddenly assumes a new position in the orbit. During the inter-flick periods the other three types of eye movements have been recorded, namely 1. an irregular tremor, probably due to the incompletely fused tetanic contractions of the extra-ocular muscles, 2. slow waves, and 3. slow drifts. These involuntary eye movements produce corresponding shifts of the retinal image of the fixated point.

The continuous retinal image movements are an essential condition for the visual function. This follows from recent experiments in which optical systems have been employed which allow the production of a stationary or stabilized retinal image in spite of these involuntary eye

movements (DITCHBURN and GINSBORG 1952, RIGGS *et al.* 1953, DITCHBURN *et al.* 1956, CORNSWEET 1956). Under these conditions the fixated test point fades out and disappears from view.

The saccadic movements or flicks are the only involuntary eye movements that are controlled by visual information (retinal feedback). In a dark room without a fixation point a steady gaze can not be maintained, and in this way the eye moves gradually farther and farther away from its initial position (DITCHBURN and GINSBORG 1953, CORNSWEET 1956). When during the fixation of a test point the image of this point moves to the edge of the central territory of the fovea, the probability increases that it will be brought back in the direction of the centre of this territory by a "return" flick (DITCHBURN and GINSBORG 1953, DRISCHEL and LANGE 1956). CORNSWEET (1956) suggests that there might exist three separated physiological mechanisms controlling the flicks. The first would determine the moment at which a flick will be performed, the second the particular direction of a given flick, and the third the particular magnitude of this flick.

In the following the involuntary mechanisms keeping the image on the fovea will be called the "preserving" fixation mechanisms in contradistinction to the visual grasp reflex mechanism, which can be initiated or inhibited voluntarily.

A smoothly moving object is followed by smooth movements of the eyes (CORNSWEET 1956), the speed of which can be modified in discrete steps whenever necessary, and upon which saccadic movements are superimposed for the correction of position errors (WESTHEIMER 1954). The control mechanisms of these smooth following movements are not yet clear. The assertion of WESTHEIMER and CONOVER (1954) that the smooth following movements can be performed without a moving stimulus source, is misleading, because they confuse the voluntary initiated rapid visual grasp reflex with the smooth following movements with which a moving object is kept on the fovea even when this object moves very slowly.

In the normal human being the visual grasp reflex can be evoked by any peripheral light stimulus: a flash of light or some strong stimulus contrast in the periphery of our field of vision attracts our gaze involuntarily, the foveas are "attracted" by the image of the stimulating source. On the other hand we can move our eyes by an act of volition and direct them on a given object. But as soon as the movement is initiated voluntarily the eyes are steered involuntarily by the fixation mechanisms in such a way that the fovea moves towards the image and remains there.

There are two widely separated cortical areas concerned in these eye movements: the frontal and the occipital oculomotor centres. Movements of the eyes in certain directions have been obtained by local electrical stimulation of these parts of the cerebral cortex. Both patho-

logical lesions and stimulation experiments indicate that the frontal centres are concerned with the voluntary eye movements while the occipital centres mediate only the lower reflex-like responses. An individual can neither move his eyes in response to a command, nor look at will at an object in any direction when the functions of the frontal centres are disturbed (for instance by injury to the projection tracts from these centres). This failure is not due to paralysis since the eyes do follow a slowly moving point, the image of which falls on the fovea. There is a tendency to persistent fixation of an object: the fixation mechanisms anchor the eyes on an object. When there are two marks on a sheet of paper the patient may be unable to turn his eyes from one point to the other, but he can often do so when the points are joint by a line. During reading the eyes travel slowly from letter to letter. The next line can only be reached by following a moving finger which leads the eyes back to the start of the next line. The fixation can only be broken by interruption of the retinal (foveal) stimulation: the patient blinks, places a hand between the eyes and the fixated object or jerks his head. When, on the other hand, the "preserving" fixation mechanisms (see p. 79) localized in the occipital oculomotor centres are disturbed by lesions of the cortico-tectal tract, the patient is unable to keep up accurate fixation: the eyes oscillate about the object he tries to fix, he is unable to keep his eyes steadily in a position to which they have been brought by voluntary contraction of the muscles.

These facts which are borrowed for the greater part from HOLMES (1938), will now be confronted with the recent data about the fixation mechanisms mentioned above, and with certain ideas about orientating movements developed in ethology. As we have seen the experiments of CORNSWEET (1956) and DRISCHEL and LANGE (1956) have shown that the saccadic movements of the preserving fixation mechanisms are controlled by visual information, received when the image of a test point moves towards the edge of the central territory of the fovea. This "image" is, of course, only characterized by a difference in stimulation intensity between some neighbouring receptor units, since the test point can be a bright spot on a dark background as well as a dark spot on a bright background. Consequently local contrasts are the only features of the image upon which the visual information underlying the releasing and steering of the saccadic movements of the preserving fixation mechanisms can be based. A similar reasoning shows that the same holds good for the visual grasp reflex. Under normal conditions these subordinated mechanisms are controlled by higher integrative levels which make possible the turning of the gaze in any desired direction, and the persistent fixation of a given object in spite of strong stimulus contrasts

in the periphery of the visual field, while the fixation of an object can be broken off at any desired moment.

With these considerations we have reached the integration levels which are studied in comparative ethology. In the following the current ethological terms (see TINBERGEN 1951, BAERENDS 1957) are used rather arbitrarily for both low-level and high-level concepts. The terms especially created for the lower levels of behaviour by RUSSELL *et al.* (1954) are not employed because they still await general acceptance.

As an example of the normal functioning of the fixation mechanisms the hunting behaviour of a predatory fish like the pike (*Esox lucius*) will be described. As soon as the drive for food has reached a certain level the first phase of hunting behaviour may be released by any object within a certain size range that moves within a certain velocity range. When this object was a suitable prey HOOGLAND *et al.* (1957) observed the following chain of activities: 1. eye movements towards the prey, 2. turning towards the prey, 3. stalking, 4. leaping, 5. snapping, 6. turning the prey head-forward, and 7. swallowing. When the prey was not entirely satisfactory, for instance in case of a stickle back, this chain could be broken off in response to visual stimuli after 1. and at every other moment if the pike had learned previously that a stickle back is not a comfortable prey.

These observations are in agreement with angler's experience that predatory fish like the pike are sometimes over-particular in the choice of their prey. On other occasions, however, they may attack almost every moving object. In all probability this is due to an increased drive for food which reduces the selectivity of the releasing mechanisms belonging to the respective phases of the hunting behaviour (releasing of the fixed pattern by suboptimal stimuli, see also TINBERGEN 1951, p. 59–63).

According to HESS (1946) and HESS *et al.* (1946) a pike does attack an inadequate prey, while a cat would not do so, because the highest visual integration centre of the pike which would steer the eyes and induce the attack is localized in the tectal region, while in a higher vertebrate like the cat the attack is controlled by higher centres localized in the cortex. The fact that the pike can break the chain of hunting activities already after mere fixation of an inadequate prey invalidates this reasoning. No doubt the control functions in question which are localized in cortical centres in the cat, are localized elsewhere in the central nervous system of the pike in a perhaps "lower" but functionally comparable form. This is, of course, only a casual example of a general rule. Otherwise the activities of a "lower" animal could not be "directive" or "purposive" and not possess the survival value

which they demonstrate when the animal is observed in its normal habitat.

Returning to the feeding behaviour of the pike, the chain of activities can be described more closely as follows: some time after the pike has taken a prey the threshold for the release of feeding activities gradually lowers, or put in other words, the readiness (drive, tendency, specific action potentiality) to perform the behaviour patterns related to feeding increases. At a certain moment a moving object which emanates a certain quantity of sign (key) stimuli releases the first fixed pattern of the chain of feeding activities: the object is fixed with the eyes. These fixation movements are steered by the stimulus contrasts, evoked in the retina by the image of the object in question. In view of the preceding description of the fixation mechanisms in man, it is reasonable to suppose that in the pike, too, the centres controlling the fixation mechanisms are of a relatively low level. As soon as the object is fixed, its configurational organization is further examined, and, when the sign stimuli necessary for the releasing of the next activity—turning towards it—are above threshold, the fixed pattern in question is released. When this is not the case, however, the chain is broken already at this moment, and the eyes return to their resting position. This reasoning can be applied to every following link of the chain of feeding activities: for the release of every link the sign stimuli received from the object—other sense organs may also be involved in the process—have to meet further requirements, and under normal conditions the result will be that the pike swallows an adequate prey.

During every phase the performed movements are the result of *a.* the running down of the released fixed pattern, which is *b.* steered by steering mechanisms reacting to the location of stimuli from the prey. As far as the eyes are concerned these steering mechanisms are servomechanisms which are controlled by retinal feedback at relatively low levels of integration. It will be evident that the motivation of the feeding instinct is a prerequisite for this chain of activities to be performed. When the object in question is not a prey, but for instance a fellow member, other instincts underlie other chains of activities.

However, when considering the behaviour of a pike in answer to an artificial light source, things seem to take another course. When the beam of light struck one eye of the pike, the following chain of activities was sometimes observed (for other activity patterns see p. 64), *a.* eye movements towards the light source, *b.* turning of the pike towards the lamp, passing into *c.* swimming towards the lamp. Frequently this resulted in a violent effort to swim through the perspex wall of the experimental tank at the point where the light beam entered.

The releasing and steering of the fixed patterns of the fixation mech-

anisms can be attributed to the image of the light source on the retina (cf p. 78). Moreover, we have seen that, as a matter of fact, the fixation mechanisms in man are released and steered by such "neutral" stimuli. But it might be expected that upon closer examination of the stimulus source no sign stimuli are received, capable of releasing further activity patterns in the pike, and as a result of this, the fixation should be broken off and the eyes should return to their resting position. Under the experimental conditions in question — the absence of other retinal stimuli — this evidently does not happen. This is a crucial point in understanding the role played by the fixation mechanisms in bringing about the trapping effect of an artificial light source. Breaking off the fixation of a given stimulus source and looking away is obviously seriously hampered when certain conditions are not fulfilled: apart from the parts of the retina receiving the stimuli which released and steer the fixation mechanisms, other parts of the retina must receive additional stimuli. Under normal illumination conditions this is guaranteed by the normal ALD.

It may be recalled that forced and persistent fixation is observed in patients suffering from disturbance of the frontal centres mediating voluntary movements of the eyes (see p. 80). In this connection some recent observations of WIESER (1956) are of interest. Patients with far advanced cerebral degenerations (apallic syndrome, KRETSCHMER 1940) and utter idiots demonstrated forced fixation of every kind of object. Moreover, when an object approached the patient up to about 70 cm he grasped at it, and at a distance of about 15 cm the object released directed gaping responses. The subjective character of the objects was of no importance: neutral (piece of cardboard), unattractive (thistle) and injurious objects (needle, knife) were fixed, grasped at and gaped at in the same way, though the patients could indicate the subjective character of these objects. It turned out that the normal configurational character of the sign stimuli, releasing the normal grasping action of the eye, the hand, and the mouth, are reduced in these patients to a very simple stimulus scheme in which only contrast, distance and movement are of importance.

After the preceding considerations about the fixation mechanisms it seems justified to conclude that, just as in the case of the pike and the lamp, these stimuli are identical with those releasing and directing the fixation mechanisms. In the normal individual these fixation mechanisms are always subordinated to higher integrative levels. A normal behaviour pattern starts with the motivation of higher centres (instincts) by internal and external factors until, for instance, the drive for food reaches a certain value. Then activity descends to lower levels. The fixation mechanisms are used during the steering of the respective

activities (in case of feeding behaviour: seeking, catching and eating food).

In the individual suffering from the apallic syndrome, as well as in the bee, the pike and the starling placed in the vicinity of a lamp in a dark room, the light stimuli start activities at low levels of coordination, without activating the superordinated centres which are indispensable for the control of the normal course of the chain of activities: the patient fixes any object and tries to grasp it, the animal fixes the lamp, turns towards it and creeps, swims or flies towards it. These activities are evidently meaningless, unpurposive, without survival value, and sometimes even leading into conditions incompatible with survival. The patient reacts inadequately because higher control centres are defective. Under these conditions lower functions act more markedly (cf. HOLMES 1938, HESS 1951). The normal animal reacts inadequately, because the presented stimulus source (the lamp) contains only sign stimuli (local contrasts) which are potent to the release of lower mechanisms (the fixation mechanisms), whereas the higher controlling centres have no opportunity to interfere. Under such extreme experimental illumination conditions strong stimuli to other sense organs (heat) which would normally not fail to release flight or escape responses, are incapable of cancelling the trapping effect of the light source effectively: a bee shrinks from the heat of a lamp, but only to return immediately until the animal is ultimately scorched (p. 58, plate I).

It seems justified to assume that the peculiar behaviour of the bees in the black test tunnel described in section IV A was the result of this drift towards the light source (the sun) and the inadequate functioning of the orientation mechanisms I and II caused by the abnormal ALD in the test tunnel.

There are some indications that even in the human being the fixation mechanisms are rather imperfectly controlled by higher centres. There are a number of geometrical illusions –the Müller-Lyer, the Zöllner, the Hering, and other illusions –which up to present have not been explained satisfactorily (cf. KOFFKA 1931, WOODWORTH and SCHLOSSBERG 1954). These illusions consist of errors in apparent length, direction or curvature in the perception of patterns of lines. In view of the recent findings about eye movements considered above, the old theory according to which eye movements could play an important part in causing these illusions, gains in attractiveness. It might be imagined that there is a continuous struggle between the "higher level" centres intending to make the eyes perform certain movements, and the visual grasp reflex and the preserving fixation mechanisms, tending to act "on their own hook" in response to accidental stimulus patterns on the retina. The stronger the accidental stimulus source is in comparison

with the object or point to be fixed, the more obvious will be the "drift" of the eyes (the foveas) from the goal towards the diverting source. This struggle might account for a great number of geometrical illusions.

Two phenomena which may be assumed to promote this drift must be mentioned: 1. "glaring", and 2. "simultaneous brightness contrast".

1. Glaring in man is ordinarily understood as the decrease in perceived brightness (the impaired visibility) of an object when an exposed light source is present in the field of view. It can be accounted for in terms of a veiling luminance produced by scattering of the light rays from the glare source in some part of their path in the eye (STILES 1929, STILES and CRAWFORD 1934, 1937, FRY and ALPERN 1953). We need not enter into the extensive literature on the subject. Recently KEIDEL (1957) demonstrated the phenomenon in the eye of a frog.

2. In the vertebrate eye it has been shown that the "receptive field of a fibre" (HARTLINE 1938) is not an independent unit of the retina, but that it is influenced by stimulation of neighbouring units (GRANIT 1952, BARLOW 1953, KUFFLER 1953). Light falling on that part of the retina which immediately surrounds a receptive field of an "on-off" unit elicits an inhibiting effect upon this unit. A similar inhibitory interaction was demonstrated by HARTLINE *et al.* (1956) with regard to the ommatidia of the compound lateral eye of *Limulus*. These interactions are thought to enhance the perceived brightness contrasts, thus improving the discrimination of objects from their background. It may be assumed that these interactions are adaptations to the illumination intensities of the objects in the animal's habitat caused by the normal ALD.

It seems reasonable to assume that the physical glaring phenomenon and the physiological phenomena underlying the simultaneous brightness contrast act together in raising the stimulus strength of an artificial light source, and in depressing the stimulus strength of other objects in the vicinity, thus promoting the drift of the eyes and of the whole organism towards the light source. In the considerations about the dazzling of a car driver by the head lights of an oncoming car this point has been overlooked. The danger of glaring lies not exclusively in the fact that the driver sees nothing but the head lights, but in the additional tendency to fix these lights and to perform the steering actions which are automatically linked up with these fixation movements: he is in danger of being victimized by the trapping effect of the head lights of the oncoming car, and he must actively steer in the opposite direction to avoid a collision.

It will be clear that the above considerations about geometrical illusion and glaring are rather tentative and that they do not at all aspire to give an exhaustive exposition of these phenomena.

IV. *The light compass reaction*

There are some examples of animals performing light compass reactions with regard to an artificial light source in the laboratory (HOFFMANN 1953, with the bird *Sturnus sturnus*, BIRUKOW 1956, and BIRUKOW and BUSCH 1957, with the insect *Velia currens*, and a number of other examples discussed on p. 24ff). In all probability the artificial illumination conditions in these experiments were "normal" enough to allow normal reactions. This can only be guessed, as exact indications with respect to the experimental illumination conditions are lacking. Moreover, light conditions which provoke abnormal reactions in one species may still be useful for normal orientation to other species.

Generally the light compass reaction can only be performed under stimulus conditions permitting the higher level control of the fixation mechanisms. In order to be able to keep the image of the sun (or the moon) in some peripheral part of the eye the bird or the insect must inhibit the fixation mechanisms from turning the eyes (and the animal) towards this strong stimulus source. Evidently this is only possible when other visual stimuli of sufficient strength are available. After the previous considerations it seems justified to assume that the higher level centres controlling the light compass mechanism and the fixation mechanisms are attuned to the normal ALD in the animal's habitat. As soon as the illumination intensity of the environment drops below a certain level in comparison with the surface brightness of the illuminating light source, the fixation mechanisms act out of control: neither the fixation of other objects, nor the performance of the light compass reaction can be achieved by the animal, and it is trapped by the light source.

C. THE TAXIS CONCEPT

Referring to the above considerations about photo orientation, some remarks may be made on the taxis concept. In order to classify the animal's reactions "to elementary physical stimuli", GUNN, KENNEDY and PIELOU (1937) modified KÜHN's classification. This resulted in the detailed classification of FRAENKEL and GUNN (1940). Here a taxis is defined as an orientated locomotory reaction leading directly towards (or away from) an undifferentiated source of stimulation. When applied to photo orientation, positive phototaxis may be defined as an orientated locomotory reaction leading directly towards an undifferentiated source of light. It will be clear that the moving of an animal towards a lamp can then easily be explained: it is quite simply a positive phototaxis. It is abundantly clear from several pages of their book that FRAENKEL and GUNN have not realized that animals, when in their habitat, never show locomotory reactions directed towards an undif-

ferentiated source of stimulation, but always towards objects with particular qualitative or quantitative characteristics. They even stated that "if telotaxis covered only such cases (reactions to light from a moving object of a particular size) we should exclude it from our system".

This is only one example of the several cases in which the "phototactic" reactions of animals under laboratory illumination conditions have been used as a basis for the elucidation of the exact nature of the light responses of animals during photic orientation under natural illumination conditions. VIAUD has still more consistently based his theories on photo orientation upon reactions under laboratory illumination conditions (p. 28–29), and even uses these reactions as a basis for peculiar psychological considerations (VIAUD 1951 a, 1951 b). Obviously he has not realized that only his "pathic" reactions *ad preferendum* represent normal orientated movements, and that his polarized "tropisms" *ad maximum* are laboratory products provoked by artificial illumination conditions.

These and other classifications of photic orientation movements are based on the predominant reaction of many animals to an artificial light source: LOEB's "forced movement". Normal photic orientation can be expected only when the experimental illumination conditions approximate the normal ALD to a degree which depends upon the experimental animal used. In general, insects are highly dependent on the normal ALD: even in a room with light coloured walls an artificial light source evokes "forced movements".

It may be assumed that in still lower organisms the normal ALD is essential for normal photic orientation movements. It might be suggested, for instance, that the abnormal experimental ALD accounts for much of the disparity experienced in imitating the diurnal vertical migration of zoöplankton, and perhaps even of phytoplankton, by phototaxis experiments in the laboratory. Here, too, it is generally taken for granted that the phototaxis observed in laboratory experiments is the result of the organism's normal orientation with regard to the presented light stimuli, a presupposition which is not justified.

In the forced movement towards a lamp we obviously have to do with a kinetic momentum causing a raised locomotory activity (see section IV and section V B). This kinetic momentum does not fit in with the current kinesis concept: the agency is not the light intensity as such, but the abormal ALD around a light source. This must be kept in mind whenever kinetic aspects of photic reactions under laboratory illumination conditions are taken into consideration.

We do not, however, go so far as to suggest that every organism must be subjected to the trapping effect of artificial light. The house

fly (*Musca domestica*), for instance, is not subjected to this trapping effect. Obviously the photic orientation mechanisms of this animal are attuned to the ALD found indoors behind a window, which is comparable to the ALD around a lamp (see p. 71). Further instances may be found in animals living on distinct light-dark boundaries (in crevices, under stones, etc.). There are several reports of animals moving away from a lamp in laboratory experiments. ANGERMANN (1957), for instance, observed that the scorpion *Euscorpius italicus* moved away from a lamp. The normal courtship and the unhampered capture of prey in blinded animals indicate that the eyes play a subordinate role. Moreover, intact scorpions paid no attention to flies behind glass. This suggests that in this case the absence of fixation mechanisms might account for the absence of the trapping effect of a lamp.

The recent tendency to discriminate between *locomotion* and *orientation* (KOEHLER 1950) is by no means new. It can be found in RADL (1903), KÜHN (1919), and others. KOEHLER'S discrimination between a locomotion component and a steering component (taxis) is analogous to the discrimination between innate automation or instinctive movement (recently called "fixed pattern") and steering component (cf. LORENZ 1937, LORENZ and TINBERGEN 1938) in the behaviour of an animal.

The recently analysed movements of the human eye (cf. CORNSWEET 1956) are particularly suitable to demonstrate that the "steering component" of an instinctive activity (some orientated movement) again can be broken up into a "fixed pattern" and a "steering component". The very small and unconscious flicks performed during fixation are the result of a "somewhere" coordinated pattern of contraction and relaxation of the eye muscles. This pattern is produced "automatically", because it continues in the dark as well as when the retinal image is stabilized, at a considerably lower frequency (vacuum activity?) and in random directions (unsteered). The flicks are normally steered by visual information derived from the location of the light stimulus on the fovea. The movements are incorporated in a feedback circuit because a non-moving stimulus source is seen at rest, while the after image is perceived in constant motion.

It does not seem recommendable to maintain the term "taxis" for the steering component of such low level fixed patterns, and so unpractical to maintain the term at all. The term "taxis" has been used not only to describe certain movements of animals, but also with reference to the underlying mechanisms. In this way the "taxis" concept has been endowed with mystical causal powers in a similar way as the "tropism" concept. It is abundantly clear from the literature that the belief in this causal power is almost unbreakably linked up with the "taxis"

concept. A term like "steering mechanism" indicates more clearly which phenomena can be attributed to its action.

D. THE APPLICATION OF THE LIGHT TRAP TECHNIQUE

It has been argued in the present paper that animals which are to be captured with a light trap must be active at night. In the majority of these animals this entails a preference for low light intensities. The ALD presented to the animals must deviate from the normal one to a degree which is characteristic of each species for the forced drift towards the light source to be provoked. The "higher" the species, the greater this deviation must be. Insects are "low" animals, and consequently they are already captured by light traps under conditions during which fishes and birds can still orient fairly normally. The drift towards the light source as compared with random movements is rather strong in insects. They can be concentrated very close to a lamp, and a light trap for insects can accordingly be small. In fishes such a strong drift towards the light can only be evoked under exceptional light conditions in the laboratory (see section IV B). During fishing with light most species of fishes show only some drift towards the light source superimposed upon their random movements, and relatively large nets are necessary to capture the fishes. Slightly turbid water as well as illumination of the bottom or nets soon tone down the abnormal ALD to a degree enabling the fish to orient normally. Then, in accordance with their preference for low light intensities, they withdraw from the illuminated locality. This might account for the contradictory results of experiments with light sources in the West European waters, especially the North Sea (p. 35-36). Similar considerations hold good for insects. Maximal catches are generally obtained with the Robinson trap. Every change in the environment of the lamp—either by other construction of the trap or by change in the locality of the lamp—which softens the abnormal ALD around the lamp by reflection of part of the light from the lamp, has generally a detrimental influence upon catches. This interferes with making reliable comparisons of faunistical or phenological data obtained by different workers with different light traps in different localities.

A word may be said about the tendency to use ever stronger lamps for catching purposes. The movements of "nocturnal" animals towards a lamp are the result of the interaction between the natural tendency to withdraw from the high light intensity in the vicinity of the lamp, and the abnormal drift towards the light source as a result of its trapping effect. According to the theory developed in the present paper it seems logical to assume that, with increasing power of a lamp, the

repellent action of the high light intensity may exceed the less increasing trapping effect. Decreasing catches will be the inevitable result (see for instance NIKONOROV 1957). The different distances at which fishes have been observed in the vicinity of lamps, too, can be explained along similar lines of thought. These distances do not simply reflect the different light optima of the respective species of fishes as has frequently been thought (see section III B). It might be imagined that the water temperature has a modifying effect upon the concentration of fish around a lamp (thermocline).

Animals can be protected against artificial light sources by toning down the abnormal ALD. In the case of birds this can be and has been achieved by additional lamps and light coloured objects in the vicinity of the main light source (the lighthouse lantern). "Lower" animals like insects are disorientated by such slight deviations from the normal ALD (see p. 71 and light vector diagram e, fig. 6 p. 70) that attraction of insects by lamps can practically only be prevented by using lamps radiating wave lengths outside the visible spectrum of insects. Because insects are sensitive to the short wave lengths far into the ultraviolet this is only possible by the use of certain longer wave lengths. The qualification "insect repellent" given recently to such lamps is, of course, pure puff.

E. MECHANICAL IMITATIONS OF LIFE

In the present paper we analysed the internal mechanisms and the external illumination conditions responsible for the transformation of an animal into a "machine" which moves "automatically" towards a lamp. On the other hand machines have been built which are supposed to exhibit some characteristic aspects of the behaviour of animals.

In the first half of the seventeenth century DESCARTES postulated the machine-like functioning and reproduction of the human body. But LOEB (1918) was the first who witnessed the realization of his mechanistic conception of life by the building of a heliotropic machine (see p. 16). DESCARTES borrowed his mechanistic conception of life from the contemporary hydraulic knowledge (see for instance HALDANE 1935), and, in the same way, LOEB used photocells and electricity for the design of the heliotropic machine. Recently, cyberneticians apply the principle of negative feedback in a similar way for the construction of electronic monstres. Already LOEB's heliotropic machine was, of course, based on the feedback principle, although at that time the term had not yet been devised.

Whether, and in how far, normal animal behaviour can be imitated by machines will not be discussed here *in extenso*, since elaborate dis-

cussions were given by SLUCKIN (1953) (cautious), de LATIL (1953) (enthusiastic), and COSSA (1955) (critical). Only some fundamental remarks will be made.

According to WALTER (1950) his "*Machina speculatrix*" illustrates "positive tropism", such as exhibited by a moth flying into a candle flame. But the artefact even goes one better than the insect, for, when the light intensity exceeds a certain value the steering mechanism makes the machine turn, and it "seeks a more gentle climate". It will be clear from the present study that the performances of his machine are as much overrated by WALTER as the complicated character of the photic orientation mechanisms in the moth is underrated. "*Machina docilis*" (WALTER 1951) possesses not only the properties of being attracted to moderate light and repelled by strong light and by obstacles. It possesses a "free will" and in addition the ability to "learn" (conditioned reflex model). The impression of reality is promoted by the quasi animal-like appearance and the names given to some of these mechanical "animals".

Attention may be drawn to the dangerous tendency among certain cyberneticians, to use anthropomorphic turns of speech like: the machine is "attracted" or "repelled", is "frightened" or "astonished", "wants" or "desires" to do something, and "enjoys" doing so, the machines "recognize" each other, etc. This is incompatible with the absence of any subjective phenomenon in the cybernetic artefacts, and presents a striking contrast to the care taken in ethology in describing the activities of real animals in terms which allow an exact measurement of the introduced concepts, without denying the existence of subjective phenomena in the animal.

No doubt the recent cybernetic robot cult results from the yielding of its supporters to the temptation to conclude from the *resemblance* between the machine and the animal to their *identity*, a fundamental mistake in biological thinking which has repeatedly been made during efforts to reduce the functioning of organisms to certain "mechanisms".

With regard to the performances of his *Machina speculatrix* with its few scarcely interconnected elements, WALTER (1950) did not hesitate to postulate "that there is no logical or experimental necessity to invoke more than number to account for our subjective conviction of freedom of will and our objective awareness of personality in our fellow men". He was sure, moreover, to be able to build in reproduction. Others have still gone further. They give serious considerations to the evolution of artefacts. Some "struggle for life" between the not quite identical products of robot self assemblage would result in gradually "better" and more "clever" machines, which would ultimately shelve their original creator (ASHBY 1948). VON NEUMANN (1951, cited by McCUL-

LOCH 1955) has proposed that the coupling of a machine with inherent learning capacities and an assembling machine would result in the production of new machines better adapted to their environment. When starting with a protein molecule as the first robot with learning capacities, McCULLOCH (1955) suggests "that it is possible to look to Man himself as a product of such an evolutionary process of developing robots, begotten of simpler robots, back to the primordial slime". Clearly Darwinism and Lamarckism are both represented in theories about robot evolution! Cyberneticians have not let out much about robot embryology (assemblage) and it seems rather difficult to misuse embryological concepts for this purpose. Curiously the biological phenomenon of extinction has not been envisaged in their considerations on robot evolution, though it might appear that robots are particularly vulnerable to this trouble.

It seems that the cybernetic conception of negative feedback is on the way to become badly overrated as an explanatory principle in biology. When exposing the re-afference principle, VON HOLST and MITTELSTAEDT (1950) emphasized that this is only one of the mechanisms, already found or yet to be found in organisms. It seems that the feedback mechanism can be looked upon as one of those features of organisms which has previously been described as regulative interactions within a mesh-work of causal relations (JORDAN 1935, 1941). When a machine exhibits feedback this is the result of our mental faculties setting up this order.

The possibility that cybernetic artefacts become mere toys is a not imaginary danger when the observation of, and the experimenting with real animals are pushed into the background. Fortunately this tendency is counterbalanced by sound efforts to analyse when, where, and in which way feedback mechanisms operate in organisms. Only some examples which are closely connected with the problem studied in the present paper will be mentioned. MITTELSTAEDT (1957) analysed the feedback mechanisms involved in photic prey localization by mantids. BASTOCK *et al.* (1954) tried to extend the range of application of the re-afference principle to higher integration levels in animals, while similar efforts were made with regard to human behaviour (Prosensory Input of HAYES *et al.* 1954).

The trapping effect of artificial light upon organisms would not have been made comprehensible by the construction of "phototropic" machines, but only by observation of and investigation into the photic orientation of organisms as described in the present paper.

VI. SUMMARY

Attempts were made to find out why insects and fishes can be captured with the help of lamps, why birds fly against lighthouse lanterns, and why in the laboratory phototaxis is preponderantly positive phototaxis.

An extensive review of the literature revealed that none of the numerous old and new theories on photic orientation can account for either of these phenomena. Analysis of the abundance of data on the trapping effect of an artificial light source upon insects, fishes and birds has led to the working hypothesis according to which the low illumination intensity of the environment around such a light source interferes with normal photic orientation resulting in a drift of the animal towards the light source. The observed concentration of animals in the vicinity of a lamp is thought to be the statistical result of this drift.

Experiments with insects (bees) demonstrated that an adequate screening of the light scattered from the sky, together with the elimination of the reflection of light by the environment really result in a disorientated drift towards the light source, even when this is the natural light source (the sun). Fishes and birds were forced to move towards a lamp under similar illumination conditions.

Photic orientation is assumed to be accomplished by the goal-directed functioning of a number of hierarchically coordinated centres. The animal's movements are controlled by optic feedback based upon the normal differences in the intensities of the light stimuli acting upon the respective photosensitive surfaces. During more detailed orientation, fixation mechanisms are put in circuit by higher coordinating centres in response to sign stimuli.

The normal values of these stimuli are determined by the normal angular light distribution in the animal's habitat, which is caused by: 1. the nature of the light sources (sun, moon, stars); 2. the scattering and absorbing capacities of the media (the atmosphere and the water); and 3. the reflecting capacity of the environment.

The abnormal feedback resulting from the abnormal angular light distribution around a lamp—brought about by the elimination of the factors 2 and 3—makes the animal deviate from the intended position or direction of locomotion. Moreover, the servomechanisms of lower coordination levels controlling the fixation movements of the eyes become a play-thing of the stimuli from the lamp that are quantitatively supernormal as compared with the adequate sign stimuli which normally activate the higher coordination centres of the fixation mechanisms. In this way these higher centres are more or less eliminated from the orientation process. Under extreme laboratory illumination conditions this results in a forced drift of the animal towards the lamp irre-

spective of factors which are incompatible with survival. Similar phenomena in human beings suffering from disturbance of the centres mediating eye movements, and in patients with far advanced cerebral degenerations (apallic syndrome) are thought to favour this concept.

The implications of the present concept of photic orientation and disorientation are discussed with regard to the current concepts of phototaxis and photokinesis, the light trap technique, some optical illusions, and glaring lights in traffic. The tendency among cyberneticians to overrate the performances of life-imitating—e.g. "phototropic"—machines, which trifle with the complexity of living organisms, is criticized.

RÉSUMÉ

Le but de la présente étude était de tenter d'expliquer pourquoi les insectes et les poissons peuvent être capturés au moyen d'une source lumineuse (lampe), pourquoi les oiseaux volent contre les lanternes des phares, et pourquoi la phototaxie que l'on observe en laboratoire est la plupart du temps positive.

Un examen approfondi de la littérature a montré qu'aucune des nombreuses théories – anciennes ou nouvelles – de l'orientation photique, ne fournissait une explication satisfaisante de ces phénomènes. En analysant les observations très nombreuses qui ont été faites sur le rôle de piège qu'une source de lumière artificielle peut jouer pour les insectes, les poissons et les oiseaux, nous avons formulé l'hypothèse de travail suivante: la faible intensité lumineuse de l'environnement, aux abords d'une source lumineuse de cette nature, interférerait avec l'orientation photique normale et résulterait en une désorientation de l'animal dans la direction de la source lumineuse. On peut supposer que la concentration d'individus que l'on observe au voisinage d'une lampe représente le résultat statistique de cette désorientation.

Des expériences sur des insectes (abeilles) ont montré qu'un filtrage adéquat de la lumière diffusée par le ciel, ainsi que l'élimination simultanée de la réflexion de la lumière par l'environnement, entraînent effectivement une désorientation dans la direction de la source lumineuse, même lorsqu'il s'agit de la source lumineuse naturelle (le soleil). Des trajectoires forcées dans la direction d'une lampe furent obtenues chez des poissons et des oiseaux dans des conditions d'éclairement similaires.

Nous supposons que l'orientation photique s'accomplit grâce au fonctionnement téléologique d'un certain nombre de centres coordonnés de façon hiérarchique. Les mouvements de l'animal sont contrôlés par un feedback optique résultant des différences normales d'intensité

lumineuse des stimuli photiques agissant sur les surfaces photosensibles respectives.

Dans le processus d'orientation plus détaillée, les mécanismes de fixation sont mis en circuit par les centres de coordination supérieurs en réponse à des stimuli-signaux.

Les valeurs normales de ces stimuli sont déterminées par la distribution angulaire normale de la lumière dans l'habitat de l'animal, laquelle est causée par: 1. la nature des sources lumineuses en cause (soleil, lune, étoiles); 2. la capacité de diffusion et d'absorption des milieux considérés (l'atmosphère et l'eau); et 3. le pouvoir réfléchissant de l'environnement.

Le feedback anormal résultant de la distribution angulaire anormale de la lumière aux abords d'une lampe – déterminé par l'élimination des facteurs 2 et 3 – fait dévier l'animal de sa position ou de sa direction de locomotion intentionnelle. En outre, les servomécanismes des niveaux inférieurs de coordination contrôlant les mouvements de fixation des yeux, deviennent le jouet des stimuli émis par la lampe, lesquels sont quantitativement supranormaux en comparaison des stimuli-signaux adéquats qui activent normalement les centres supérieurs de coordination des mécanismes de fixation. De cette façon, les centres supérieurs sont plus ou moins éliminés du processus d'orientation. Ceci entraîne, dans des conditions extrêmes d'éclairement réalisées en laboratoire, une dérive forcée de l'animal vers la lampe, même si des facteurs incompatibles avec la survie interviennent à ce moment. Des phénomènes similaires constatés chez des sujets humains souffrant de troubles des centres commandant les mouvements des yeux ou atteints de dégénérescences cérébrales avancées (syndrome apallique) étayeraient à leur façon la théorie que nous proposons ici.

Les implications de la présente conception de l'orientation et de la désorientation photiques sont discutées en relation avec les conceptions courantes de la phototaxie et de la photocinèse, de la technique des pièges lumineux ainsi que de quelques illusions d'optique et de l'éblouissement dans le trafic.

La tendance des cybernéticiens à surestimer les performances des machines imitant l'activité des organismes vivants (par exemple le "phototropisme"), et qui semblent se jouer de la complexité de ces derniers, est également critiquée.

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DISTRIBUTION AND SEASONAL ACTIVITY IN THREE SPECIES OF DIPLOPODS

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I. INTRODUCTION

Until relatively recently, diplopods have attracted the interest of zoologists only from the viewpoint of their taxonomy. With the realization that serveral species may, under some circumstances, become injurious to crops, a modicum of interest was aroused in their ecology. Up to that time, it seemed generally to have been assumed that diplopods, as a group, were more or less homogeneous in their ecological requirements, that they inhabited damp places and were very susceptible to the effects of desiccation. Recent comparative investigations by PERTTUNEN (1953) and BARLOW (1957) have indicated, however, that considerable differences may exist in behaviour and ecology between closely related species.

It is the purpose of the present paper to present a comparative analysis of the distributions and seasonal activities of three species of millipedes: *Polydesmus denticulatus* C. Koch, *Brachyiulus littoralis* (Verh.), and *Cylindroiulus frisius* (Verh.), which occur in sand dunes along the west coast of Holland. This work is part of an extensive study of the ecology of dune animals which was begun at this laboratory in 1953.

II. METHOD

Sampling of populations, in this work, is achieved by a method of trapping using, as traps, metal boxes with dimensions of 24 × 24 × 27 cm, which are buried in the ground up to the top rims. A screen-covered hole in the bottom of each box provides for drainage of rainwater. To reduce the amount of water, leaves, sand, etc., which tends

to collect in the traps, metal roofs of 30 × 30 cm. are placed over them. The covers stand on metal legs which hold them several cm. above the surface of the ground. Experiments have indicated that these roofs do not influence the catch.

One hundred such traps are placed in groups of three or six in various types of terrain from bare sand quite near the sea, to relatively old, dense woods, with various biotopes intermediate in succession between these extremes. The following is a description of these different terrains, on a macrobiotope level.

Series 1.—Very rough, relatively bare dunes with one or two groups of small trees; comparatively near the sea:

a. six traps in largely bare sand (biotopes 1 and 2);
b. six traps in a depression carpeted by mosses, lichens, and short grass with here and there a few creeping willows (*Salix repens* L.) (3 and 4);
c. three traps in each of two small woods consisting of small birches (*Betula verrucosa* Ehrh.) and situated relatively near the sea (5 and 8);
d. three traps outside each of the woods in *c.*; three in bare sand with patches of the grass *Ammophila arenaria* (L.) Link., (6), and three in a thick growth of *Calamagrostis epigejos* (L.) Roth., (7).

Series 2.—Similar to series 1 but farther from the sea. Largely bare sand with a few groups of small trees or bushes;

a. on a plain thickly covered with mosses, lichens, and short grass (21);
b. in bare sand (22);
c. in a growth of *C. epigejos* under scattered birches (23);
d. under creeping willows (24);
e. on a hillside among a number of old black poplars (*Populus nigra* L.) (25).

Series 3.—Woods situated in a steep-walled depression among rough terrain. About equal distance from the sea as series 2:

a. on a small plain overgrown by *C. epigejos* (26);
b. two groups of three traps each in dense birch woods (27 and 28);
c. four traps in an open terrain outside the wooded area in *b.*—sand overgrown by mosses, lichens and short grass with a few creeping willows (29, 34);
d. six traps on an open plateau formed by excavated sand and covered by *A. arenaria* and *C. epigejos*; about four m. higher than the woods in *b.*, (30 and 31);
e. in moist birch woods with a rich and varied undergrowth of such plants as *Mentha aquatica* L., *Valeriana officinalis* L., and *Eupatorium cannabinum* L., (32);
f. in isolated, dense, birch woods (33).

Series 4.—A relatively old wooded terrain in a completely flat area of dunes lying about two km. from the sea:

a. on a small plain, three traps in a stretch of largely bare sand (10), and three in an area partly overgrown by *A. arenaria* (9);
b. in dense aspen woods (*Populus tremula* L.), (11);
c. in open terrain covered by a dense growth of *C. epigejos* (12);
d. largely open terrain overgrown by *C. epigejos* and *Hypericum perforatum* L., with scattered birches and *Crataegus monogyna* Jacq. (13);
e. among a patch of thorn bushes, *Hippophaë rhamnoides* L. (14);

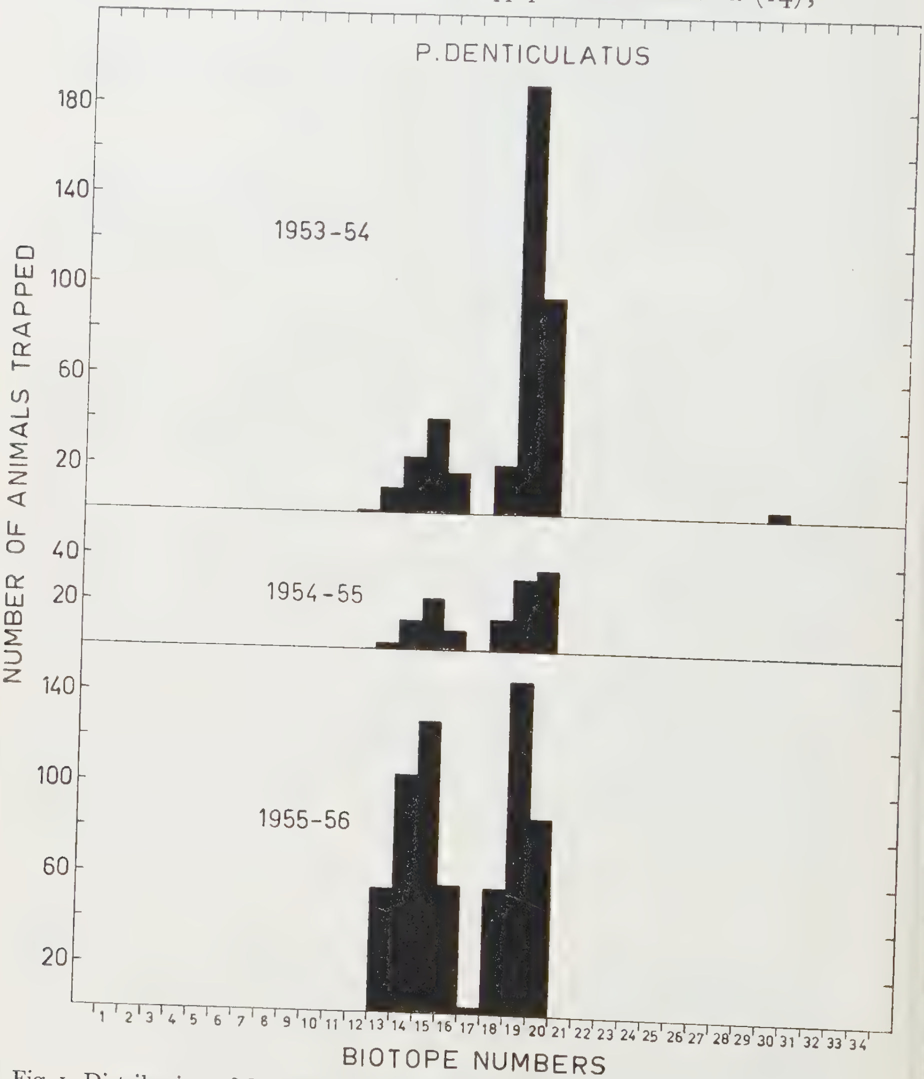

Fig. 1. Distribution of *P. denticulatus*. Total number of animals trapped per year in different biotopes. For description of biotopes, see text.

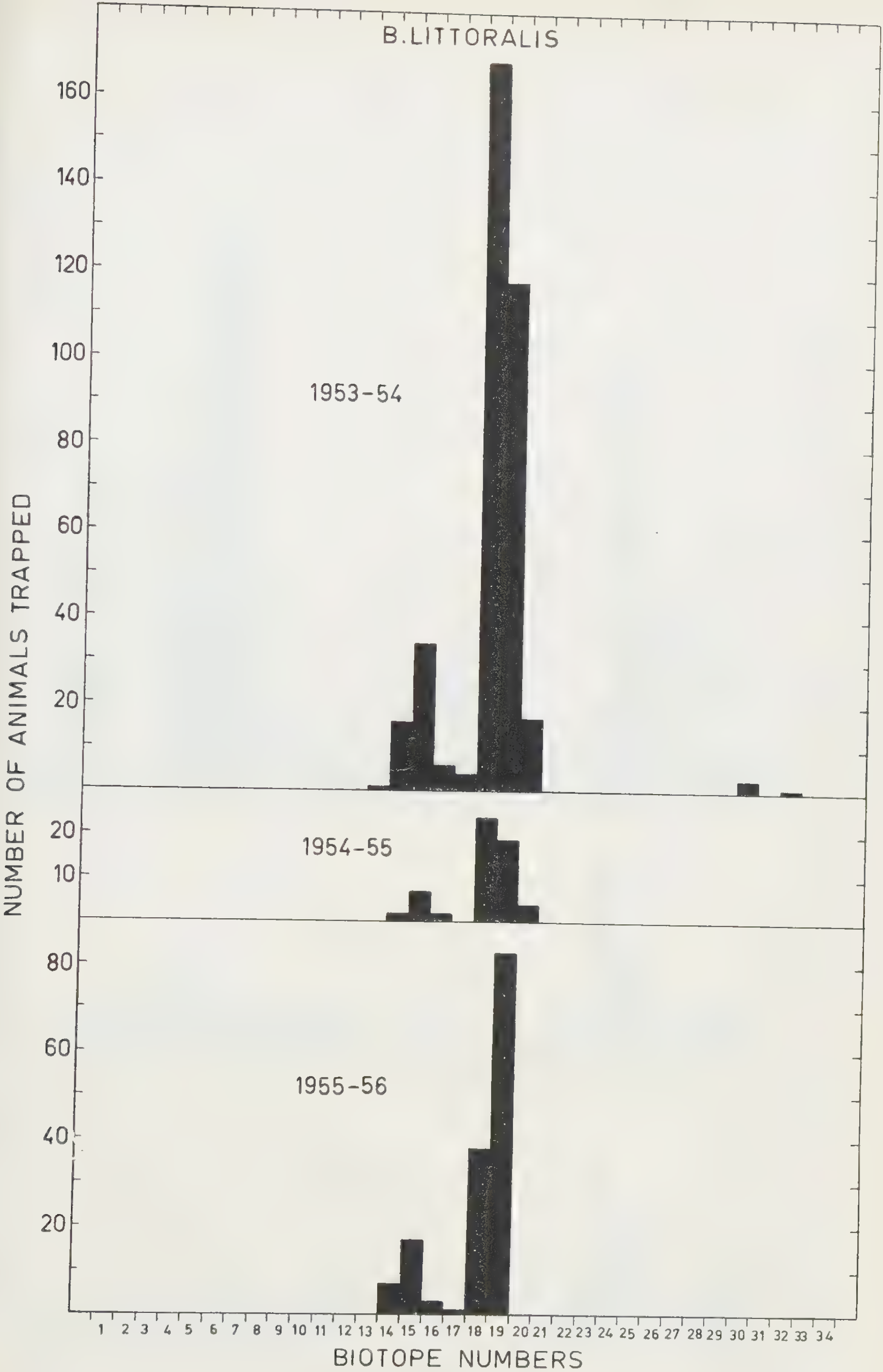

Fig. 2. Distribution of *B. littoralis*. Total number of animals trapped per year in different biotopes. For description of biotopes, see text.

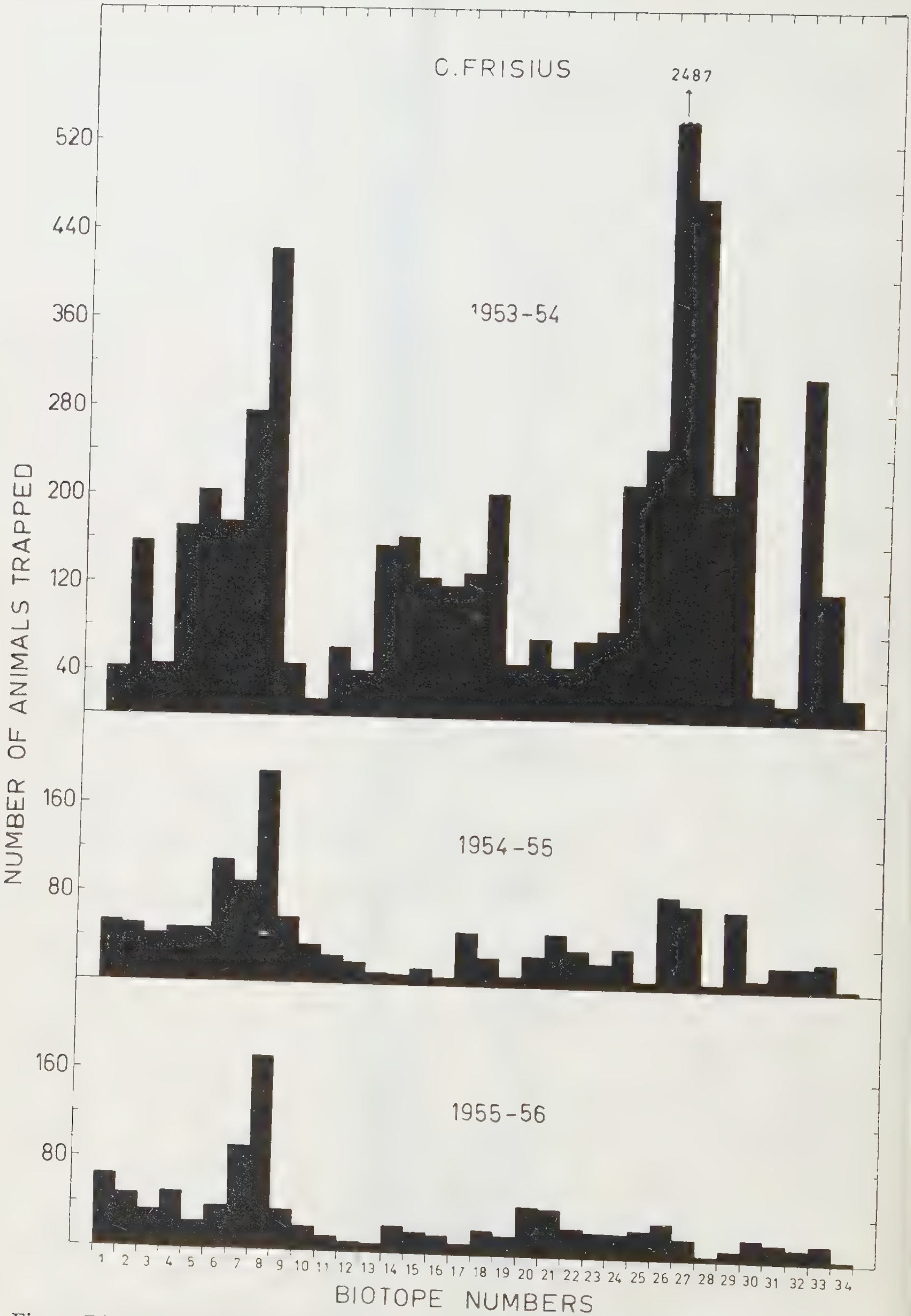

Fig. 3. Distribution of *C. frisius*. Total number of animals trapped per year in different biotopes. For description of biotopes, see text.

f. in a stand of aspen trees with a thick undergrowth of nettles, *Urtica dioica* L. (15);
g. a flat area densely overgrown by *C. epigejos* with scattered birches merging into the woods in *f.* (16);
h. a clearing in *f.* covered by *C. epigejos* (17);
i. in an extension of *f.* consisting of aspen trees with a thick undergrowth of *Humulus lupulus* L., and honeysuckle, *Lonicera periclymenum* L. (18);
j. a wooded area in the same complex and similar to *i.* but with a more varied undergrowth; three traps in a depression (19) and three on ground about two m. higher (20).

The traps are emptied once each week and the animals preserved in alcohol for later examination. Results obtained by this method reveal chiefly two aspects of the population sampled. Comparing the number of animals of each species which are caught in different biotopes indicates their relative distribution in the experimental area. But the catch is also dependent on the activity of the animals. This activity, in turn, is determined by the characteristic alacrity of the species and is modified primarily by environmental factors external to the animal and secondarily by internal factors such as hibernation and reproductive periodicity. Data collected by this method of sampling thus indicate not only biotope preferences but also the influence of environmental factors on activity as well as cyclic changes in activity during the year.

Weather conditions quoted in the analysis were derived from daily weather reports issued by a station of the government weather bureau situated a few km from the test area.

III. DISTRIBUTION

The total number of animals of each species which was trapped per year in each biotope during three successive years is shown in Figs. 1–3. The distribution of each species in the experimental area was similar in different years.

P. denticulatus and *B. littoralis*

P. denticulatus and *B. littoralis* have almost identical distributions in the experimental terrain. Both appear to be stenotopic species, closely restricted in range to woods with rich and varied undergrowth or to areas thickly overgrown with grass. Such surroundings with rich vegatative cover undoubtedly have a tempering influence on extreme temperature and present numerous microbiotopes in which both humidity of the air and moisture of the ground are usually higher than in more open areas. It is possible that these species which are so exclusively

limited to this type of terrain are sensitive to changes in environmental factors and are unable to effectively withstand conditions of temperature and humidity existing in more exposed terrain. SCHUBART (1934), however, described *P. denticulatus* as being of a somewhat xerophilic character. If this is true, factors other than humidity requirements must be responsible for restriction of this species to wooded terrain in the dunes. Laboratory experiments will be required to clarify these relationships.

C. frisius

This is a eurytopic species which occurs in all of the biotopes sampled in the experimental terrain. Generally, the greatest numbers of animals are found in wooded areas or in surrounding grass land but the range of the species extends also, although with less frequency, to more exposed terrain, sometimes to nearly bare sand (biotopes 6, 10, 22). It follows that this species is less restricted by environmental conditions than *P. denticulatus* and *B. littoralis* and is probably able to withstand more extreme temperatures and lower humidities than these more confined species. The ability of *C. frisius* to exist in areas where environmental conditions are more variable does not necessarily mean that individuals of this species are any less perceptive of environmental factors than stenotopic species. On the contrary, adaptability to a variety of conditions may involve an augmented responsiveness to environmental factors and a superior agility which enable the animal to avoid potentially injurious conditions, as with the millipede, *Iulus scandinavius* Latz. (BARLOW, l.c.). Here also, laboratory experiments are necessary to disclose whether the eurytopism of *C. frisius* is a manifestation of a similar behavioural adaptation or of physiological resistance to unfavourable conditions. It is interesting to note that this species inhabits wooded areas in association with the closely related species, *Cylindroiulus silvarum* (Mein.). The latter species, however, is entirely restricted to wooded terrain, being susceptible to desiccation and lacking the required level of activity to maintain itself in more open areas where active avoidance of dry conditions would be necessary (BARLOW, l.c.).

C. frisius also has a much more extensive geographic distribution than either *P. denticulatus* or *B. littoralis* according to SCHUBART (l.c.) who accredited this wide range to the superior adaptability of the species.

It is possible that food preferences may play some part in the distributions of these three species but this is unlikely since millipedes appear generally to be almost omni-vegetarian and seldom show pronounced preferences for particular substances as food (LATZEL, 1884; BRADE-BIRKS, 1930; CLOUDSLEY-THOMPSON, 1950; KINKEL, 1955; BARLOW, l.c.). Predation by other animals is also a negligible factor in the ecology of diplopods; they apparently have very few natural enemies.

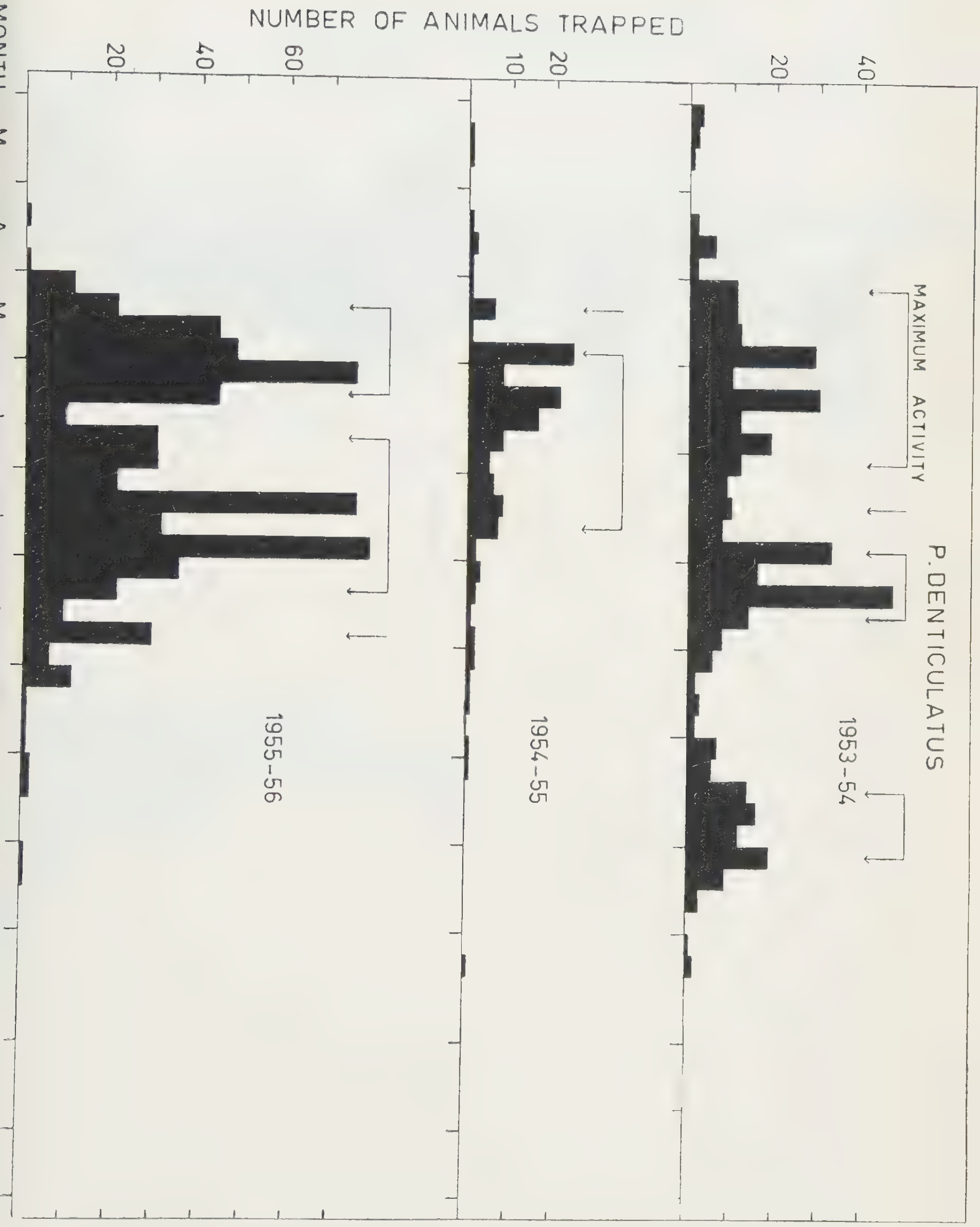

Fig. 4. Seasonal activity of *P. denticulatus*. Total number of animals trapped per week in all biotopes. Maximum activity = any number of animals trapped > mean catch per week + 1 × S.E. of the mean.

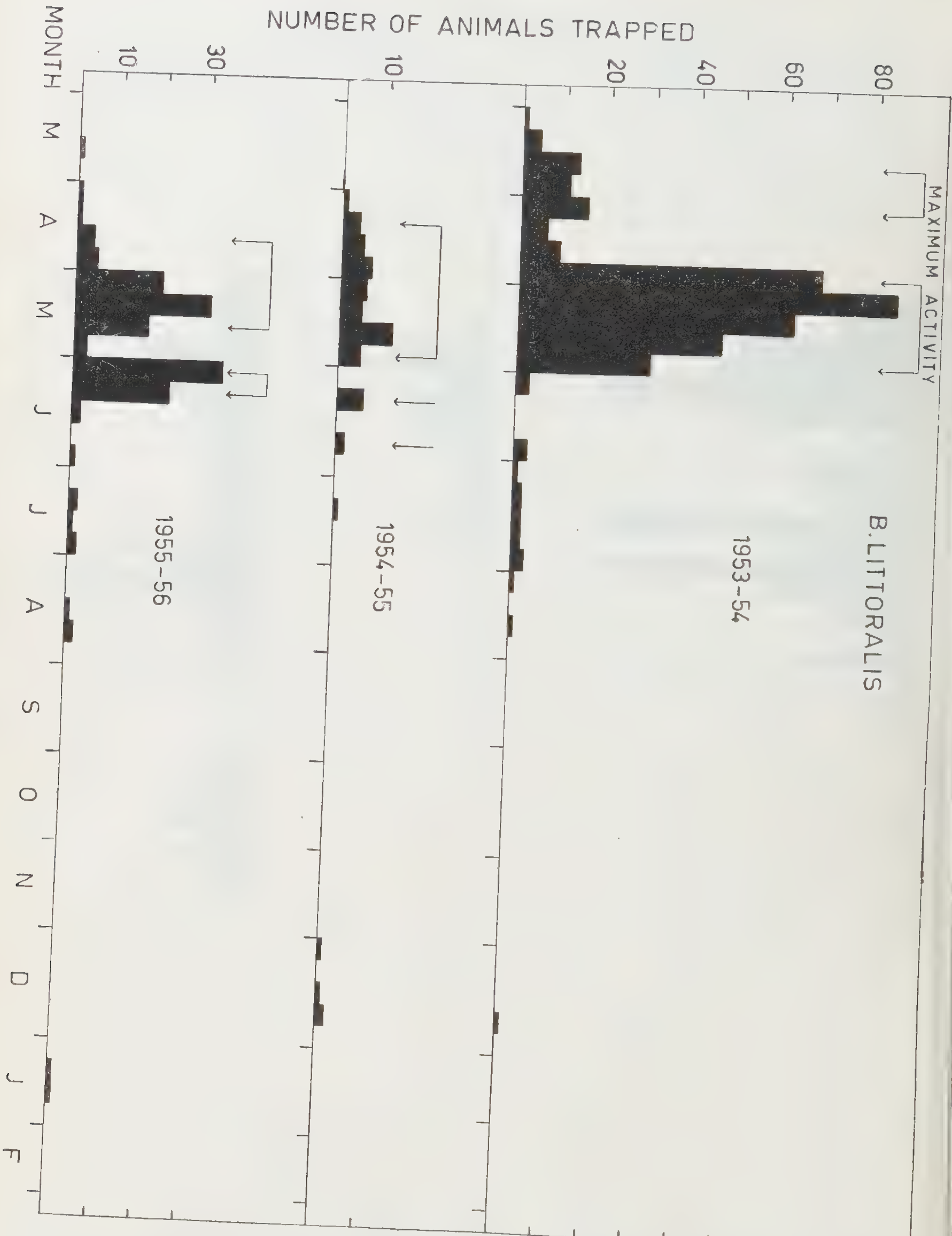

Fig. 5. Seasonal activity of *B. littoralis*. Total number of animals trapped per week in all biotopes. Maximum activity = any number of animals trapped > mean catch per week + 1 × S.E. of the mean.

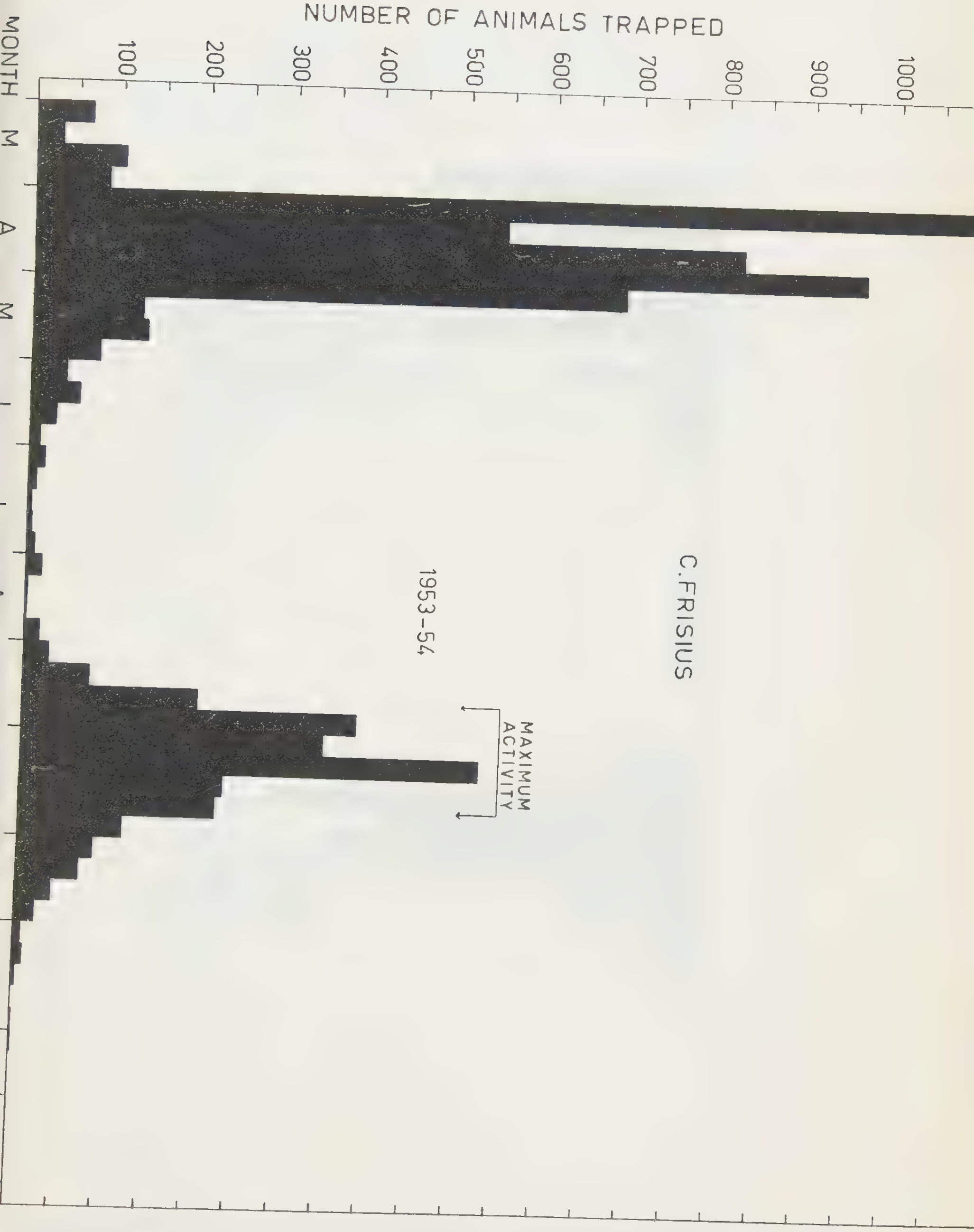

Fig. 6. Seasonal activity of *C. frisius* during 1953–54. Total number of animals trapped per week in all biotopes. Maximum activity = any number of animals trapped > mean catch per week + 1 × S.E. of the mean.

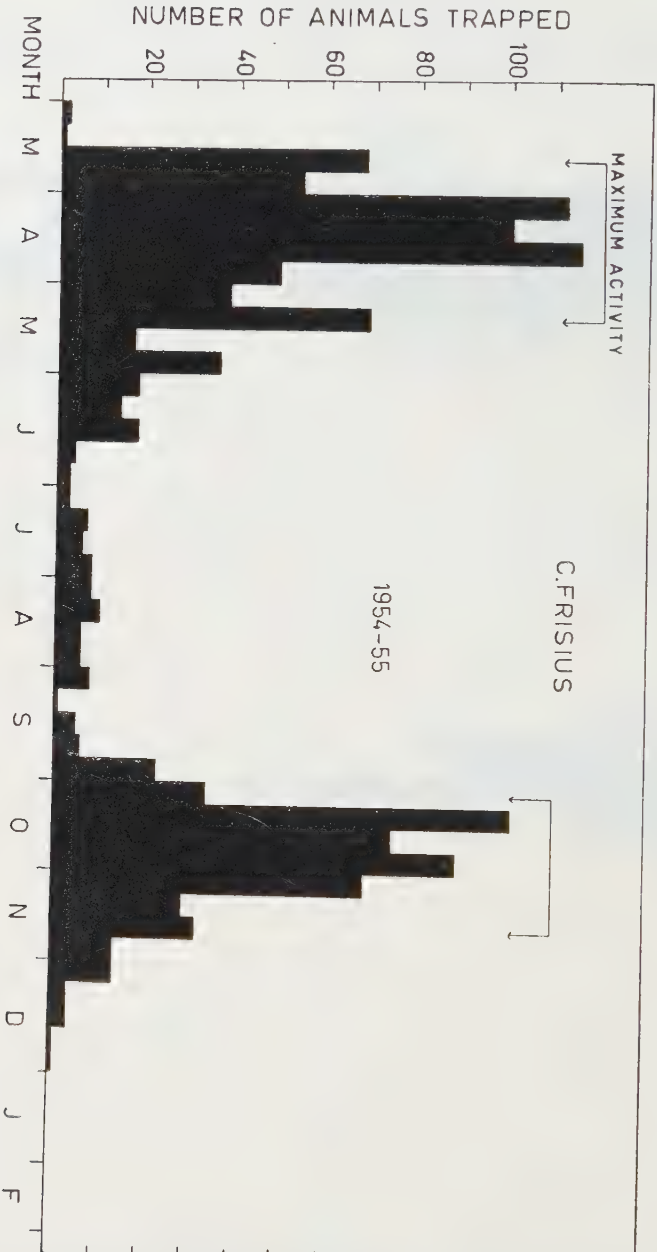

Fig. 7. Seasonal activity of *C. frisius* during 1954–55. Total number of animals trapped per week in all biotopes. Maximum activity = any number of animals trapped > mean catch per week + 1 × S.E. of the mean.

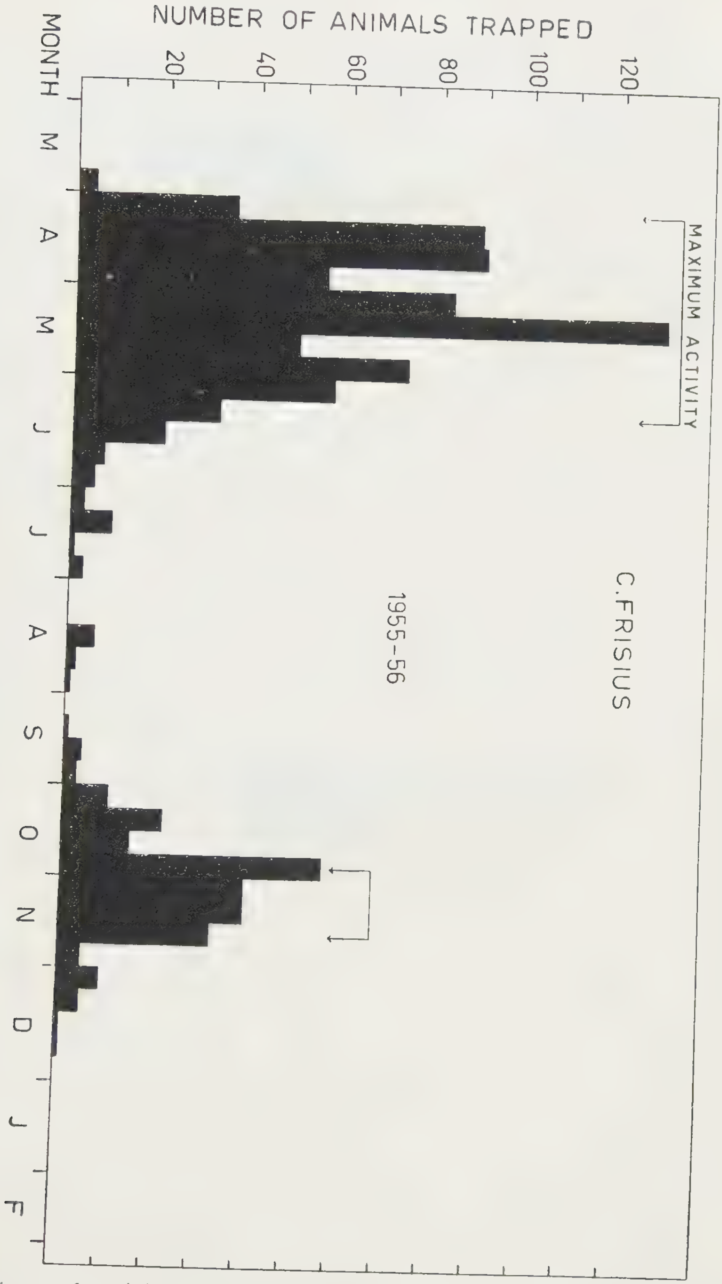

Fig. 8. Seasonal activity of *C. frisius* during 1955–56. Total number of animals trapped per week in all biotopes. Maximum activity = any number of animals trapped > mean catch per week + 1 × S.E. of the mean.

IV. SEASONAL ACTIVITY

In figs. 4–8 the total number of individuals of each species which was trapped per week in all biotopes tested during three consecutive years is plotted. All three species become active in March but the lengths of time during which they remain more or less continuously active vary. *C. frisius* is inactive only during January and February, *P. denticulatus* is inactive from about November or December until March, and *B. littoralis* shows very little activity between September and March.

Considering activity over the whole year, there are also more or less clearly defined periods during the year when the species are exceptionally active. Periodic increases in the number of young animals during the year may reasonably be reflected in sudden increments in the number of animals trapped but these increases are probably of little influence because of the slowness of growth of the millipedes and because the young animals are relatively much less active than more mature individuals. The fluctuations in numbers shown in the graphs are probably valid indications of corresponding activity changes.

To obtain a standard definition of activity maxima, the mean catch per week was calculated for each year and a maximum was then taken as any number greater than the mean per week plus one standard error of the mean. It should be emphasized that the means, in many of these cases, have little value as measurements of central tendency because of the irregularity of the distributions during the year. Means and standard errors are used only as indications of the time of year when the greatest activity was exhibited.

As with distribution, there is an essential difference between the seasonal activities of *P. denticulatus* and *B. littoralis* on the one hand, and *C. frisius* on the other hand. Maximum activity of *P. denticulatus* extends usually from May to July and sometimes into August. In *B. littoralis*, the activity period occurs earlier in the year, usually in April and May. Occurrence of maxima in activity of both these species is relatively irregular compared with *C. frisius* (figs. 6–8). This species is characterized by two, more clearly distinguishable, activity peaks during the year. The first peak, which is the largest, occurs in March and April and sometimes also in May. The second period of activity extends, generally, from the beginning of October to the first half of November. Between these periods of increased activity is a period of relative inactivity during summer. This activity pattern is also very similar to that of the closely related species *C. silvarum* (BARLOW, l.c.).

In order to examine these activity periods more closely in relation to the temperature and precipitation which existed at the time, hydrothermographs (figs. 9–14) have been constructed for the data. The

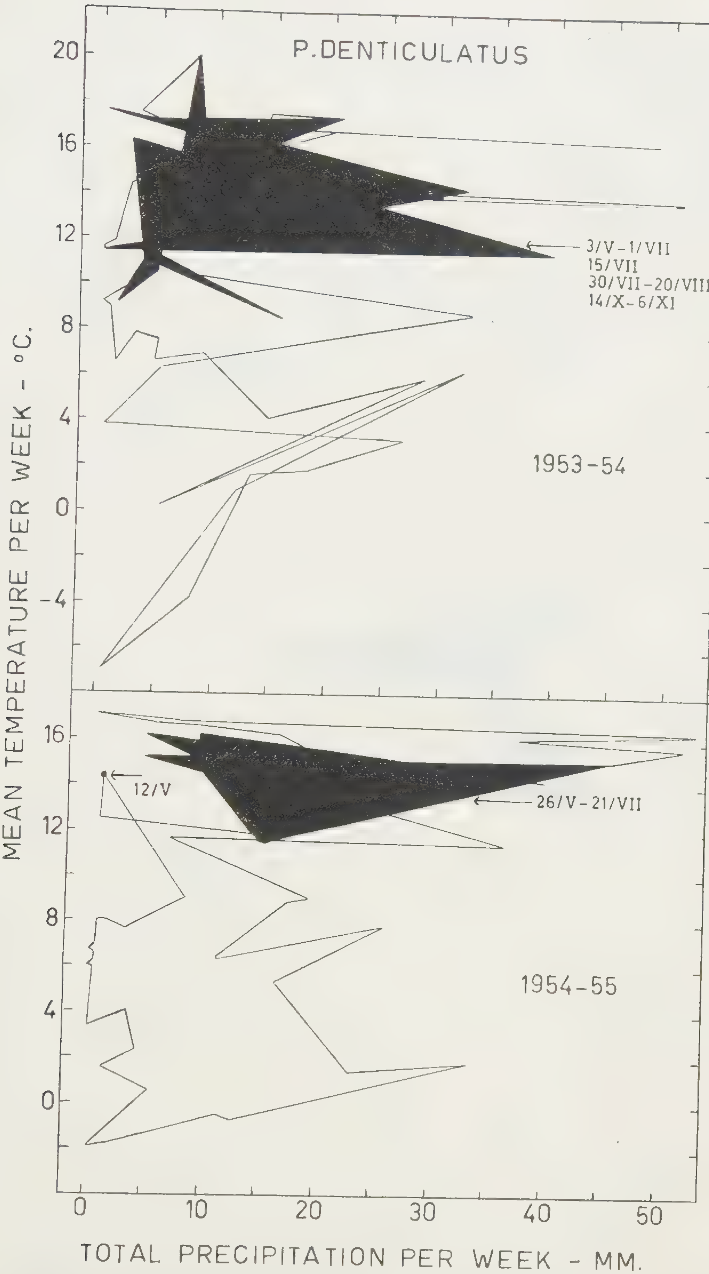

Fig. 9. Conditions of temperature and precipitation during 1953–54 and 1954–55 with periods of maximum activity of *P. denticulatus* in black, c.f., fig. 4.

mean temperature per week, calculated from recordings at three-hour intervals, and total precipitation per week were plotted together for each year and the points were joined in temporal succession. The resulting enclosed areas give an impression of the conditions of temperature and precipitation which existed, as well as the magnitudes of changes which occurred in both these factors during the year. Periods in which maximum activity occurred are indicated by black or lined areas of the figures. Each period of maximum activity of *C. frisius* was plotted separately from conditions during the rest of the year to better emphasize the regularity of occurrence and distinctness of these periods in the case of this species.

P. denticulatus

Peaks in activity of *P. denticulatus* during the years 1953–55 (figs. 9 and 10) were associated with a relatively wide range of temperature but

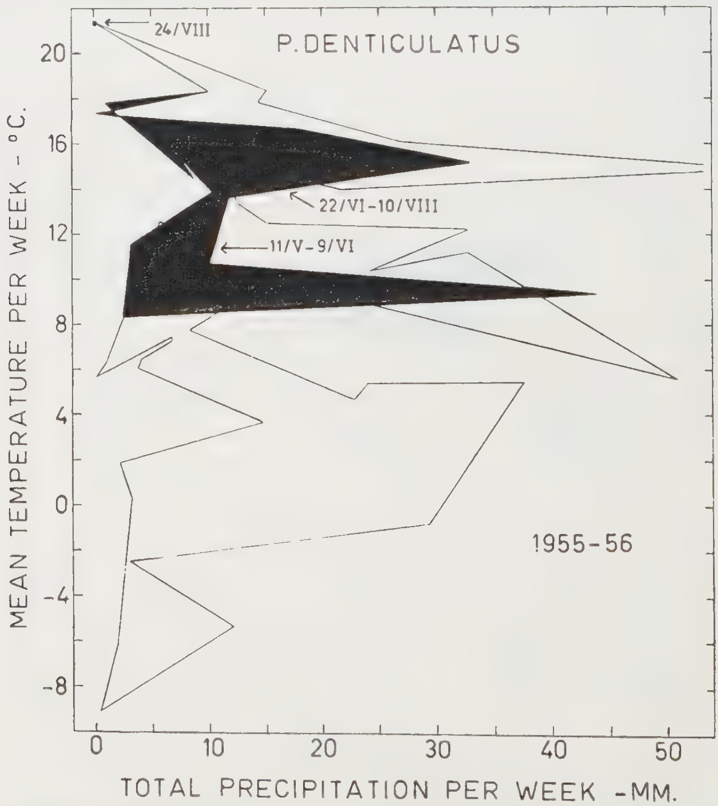

Fig. 10. Conditions of temperature and precipitation during 1955–56 with periods of maximum activity of *P. denticulatus* in black, c.f., fig. 4.

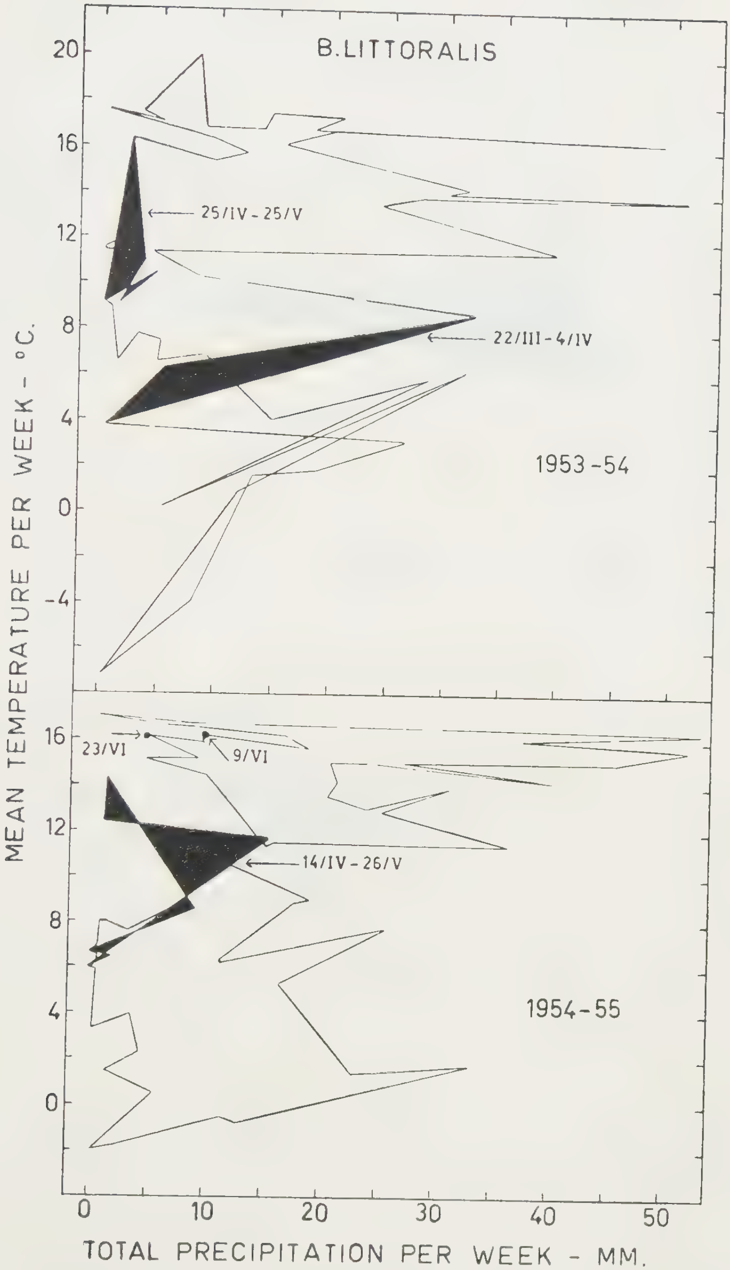

Fig. 11. Conditions of temperature and precipitation during 1953–54 and 1954–55 with periods of maximum activity of *B. littoralis* in black, c.f., fig. 5.

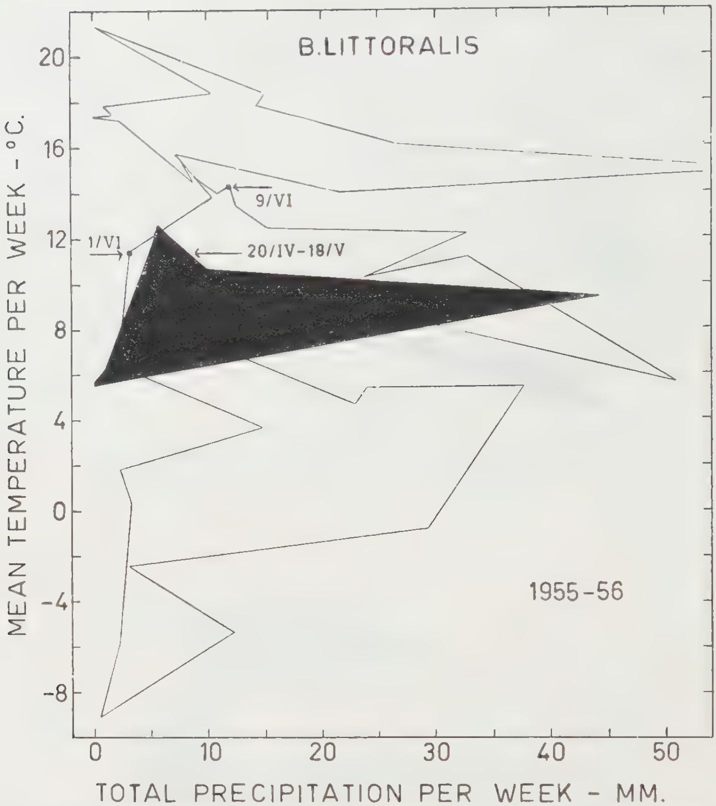

Fig. 12. Conditions of temperature and precipitation during 1955–56 with period of maximum activity of *B. littoralis* in black, c.f., fig. 5.

also particularly of precipitation. Maximum activity occurred during periods when the mean temperature per week was between about 9° and 18° or 20°. There was some difference from year to year, however. During 1954 the range of temperature at which peak activity occurred was particularly narrow.

B. littoralis

Figs. 11 and 12 show that somewhat lower temperatures and often less rainfall are typical of periods of peak activity of *B. littoralis*. This is to be expected since activity maxima of this species during spring are of shorter duration and occur somewhat earlier than those of *P. denticulatus*.

C. frisius

The presence, in *C. frisius*, of two periods of maximum activity during the year offers an opportunity of comparing conditions of tem-

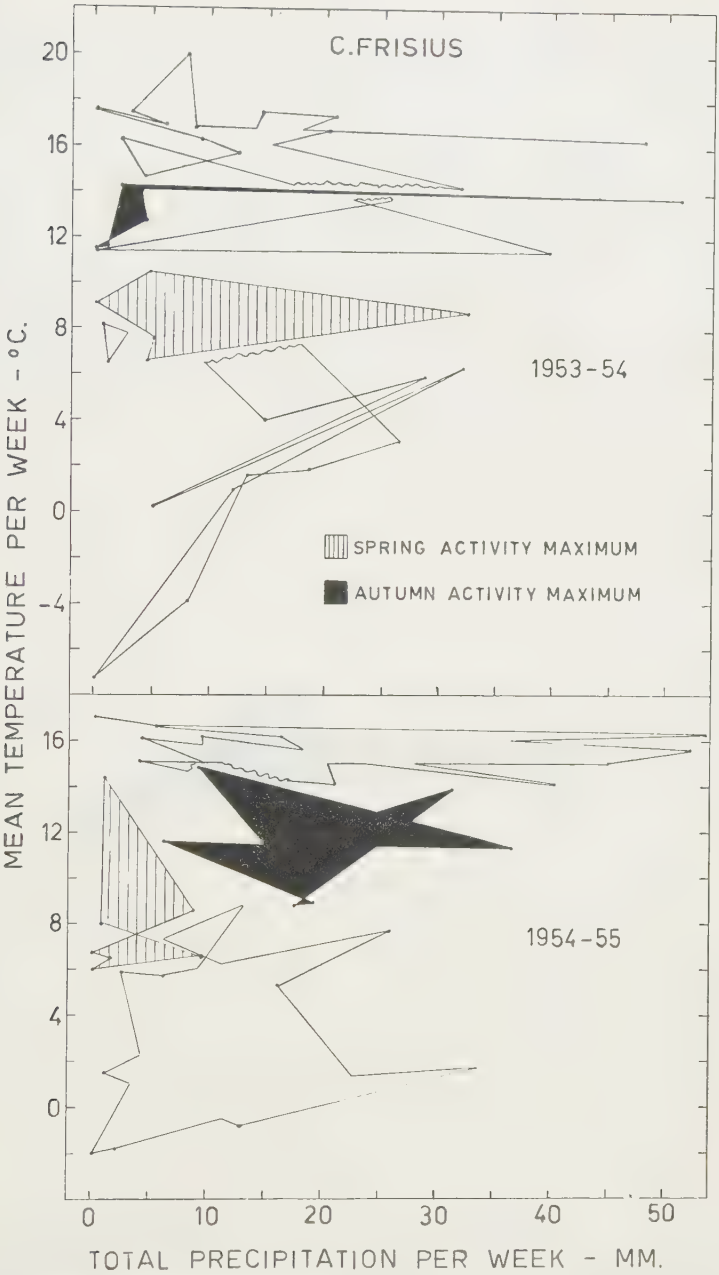

Fig. 13. Conditions of temperature and precipitation during periods of maximum activity (black or lined) of *C. frisius* and the remainder of the year (blank), c.f., figs. 6 and 7.

perature and precipitation existing during both these periods. Figs. 13 and 14 show that both spring and autumn peaks in activity of this species are associated with moderate temperature (6°–14°) and low (15—mm) to moderate (15 mm–35 mm) but sometimes also high (35 + mm) rainfall. In 1955 these two periods corresponded almost exactly, relative to temperature and precipitation. In other years autumn activity was characteristic during slightly higher temperatures and higher precipitation. Inactivity during summer usually occurred concomitant with relatively high temperature and low to high rainfall while low to moderate precipitation and low temperature were characteristic of winter inactivity.It appears that maximum activity is correlated with a definite range of temperature and that the autumn period of activity may occur in the higher temperatures of this range. This may

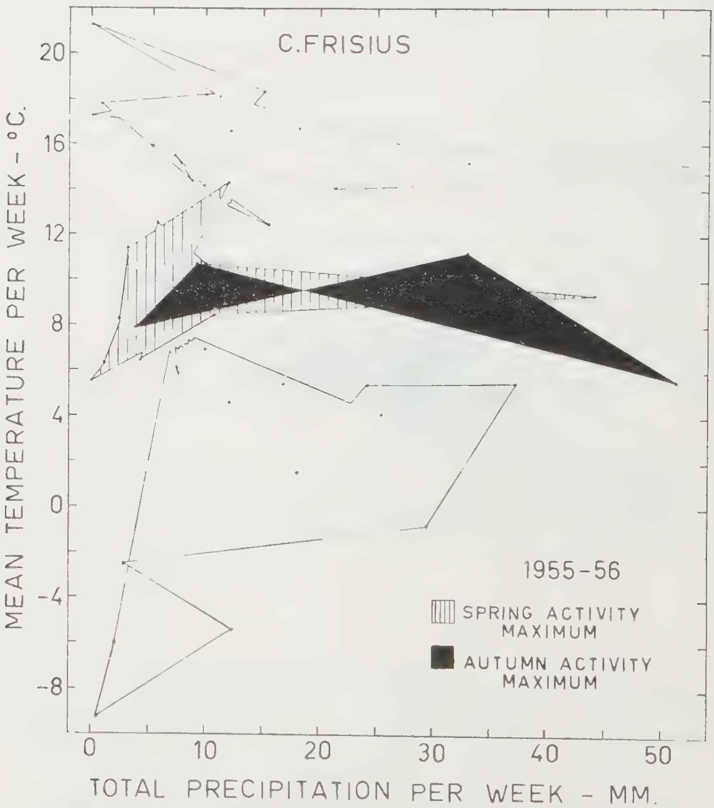

Fig. 14. Conditions of temperature and precipitation during periods of maximum activity (black or lined) of *C. frisius* and the remainder of the year (blank), c.f., fig. 8.

result from a possible difference in influence between rising temperatures (spring) and falling temperatures (autumn).

In all three species seasonal activity seems to be more a question of temperature than of precipitation. The positions of maxima in figs. 9–14 are arranged more definitely according to temperature than to precipitation both in considering any one year and in comparing different years. Periods of maximum activity are usually characteristic of a relatively wider range of precipitation than of temperature and are generally more variable from year to year in relation to simultaneous conditions of rainfall than in relation to temperature. This is logical of course if one considers the nature of changes in temperature and precipitation during the year. Seasonal changes in temperature consist of long and steady trends, rising in spring to a maximum in summer and then falling to a minimum during winter. Although precipitation also shows trends, tending to be highest, in this area, in July and August and lowest in winter, fluctuations in precipitation are more radical, sometimes being as much as 40 mm from one week to the next. But it is not merely logical that occurrence of peaks in activity of these species shows some regularity from year to year in relation to the prevailing temperature. This plus the fact that the ranges of temperature over which maxima in activity occur are characteristic and somewhat different for each species suggest a connection between temperature and activity which is more important than the possible influence of precipitation on activity. It is possible, of course, that factors other than temperature are primarily responsible for the occurrence of periods of increased activity. The appearance of two peaks in activity per year in the case of *C. frisius* may be caused by the existence of two reproductive cycles per year in this species. It is also clear from the activity periods of *P. denticulatus* and *B. littoralis* that the occurrence of these peaks, at least in these two species, does not result solely from the existence at any time of certain conditions of temperature and precipitation. Only in the case of *C. frisius* do approximately the same conditions of temperature and precipitation occurring at two different times of year correspond with the incidence of activity maxima both times.

V. INITIATION OF ACTIVITY INCREASES AND DECREASES

Occurrence of activity maxima during the year apparently bears some relation to conditions of temperature and precipitation. It is reasonable that these factors may also play some part in initiation of activity increases during spring and termination of activity later in the season. In this case, correlations might be expected between temperature and activity or precipitation and activity or between both temperature and

precipitation and activity. Table 1 shows the results of applying the rank correlation method of KENDALL (1955) using the data of figs. 4–8. In this analysis, the numbers of animals trapped during increases at the beginning of the yearly cycle and during decreases towards the end of each activity peak were compared with co-existent conditions of temperature and rainfall. Periods of increases and periods of decreases were handled separately because cycles in activity may be partly positively and partly negatively correlated with contemporary fluctuations in weather. Data for all three years were considered together. Fluctuations which occurred during maximum activity were not included.

Only with *C. frisius* were conclusive correlations regularly obtained. Increases and decreases in activity of this species were all correlated very significantly with temperature. Considering the signs of the coefficients, it appears that activity of this species increases during spring as temperature increases; as temperature continues to increase, activity declines. When temperature decreases in late summer and in autumn, activity again increases and as temperature decreases still further in late autumn, the numbers again decline. Results with *P. denticulatus* and *B. littoralis* show less conclusive relationships. Decreases in numbers of *P. denticulatus* were significantly correlated with temperature but increases of this species during spring were apparently related to contemporary increases in rainfall. Coefficients in the case of *B. littoralis* were not significant.

A significant negative correlation between activity and precipitation was also found for decreases in activity of *C. frisius* during spring. How-

TABLE I

Correlation between changes in activity and temperature and precipitation during 1953–55; T_t = rank correlation coefficient of temperature; T_p = rank correlation coefficint of precipitation

Period of activity	T_t	p	T_p	p
	P. denticulatus			
Spring, increases	0.271	0.180	0.414	0.038
Summer-autumn, decreases	0.411	0.017	0.002	—
	B. littoralis			
Spring, increases	0.297	0.177	—0.171	0.529
Spring, decreases	—0.310	0.139	—0.363	0.080
	C. frisius			
Spring, increases	0.632	0.003	0.416	0.057
Spring, decreases	—0.509	0.0003	—0.324	0.021
Autumn, increases	—0.617	0.0003	0.072	0.280
Autumn, decreases	0.559	0.0001	0.019	0.889

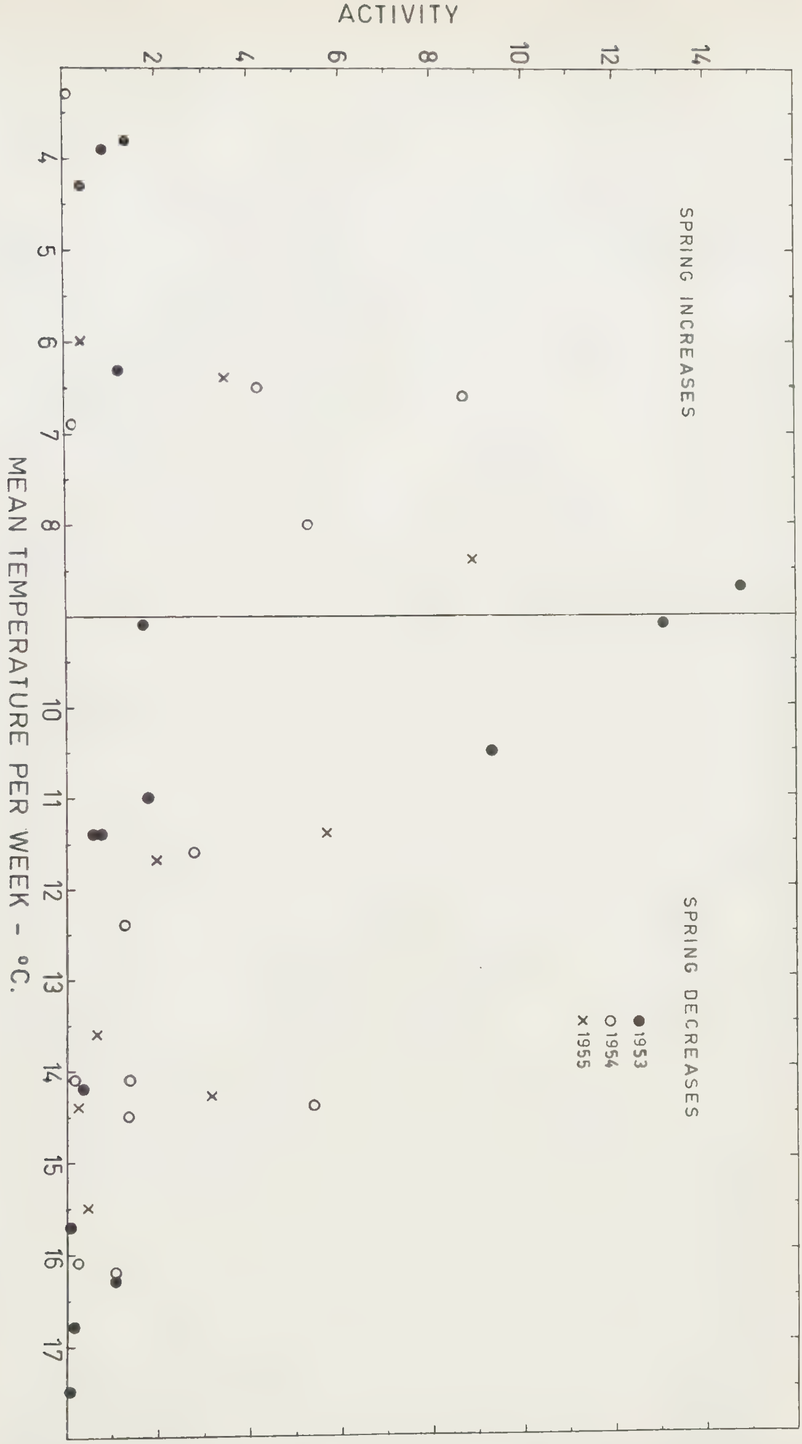

Fig. 15. Activity of *C. frisius* vs. mean temperature per week during successive weeks in which increases or decreases in the number of animals trapped were observed (c.f. figs. 6–8). Activity = number of animals trapped as % of the yearly total.

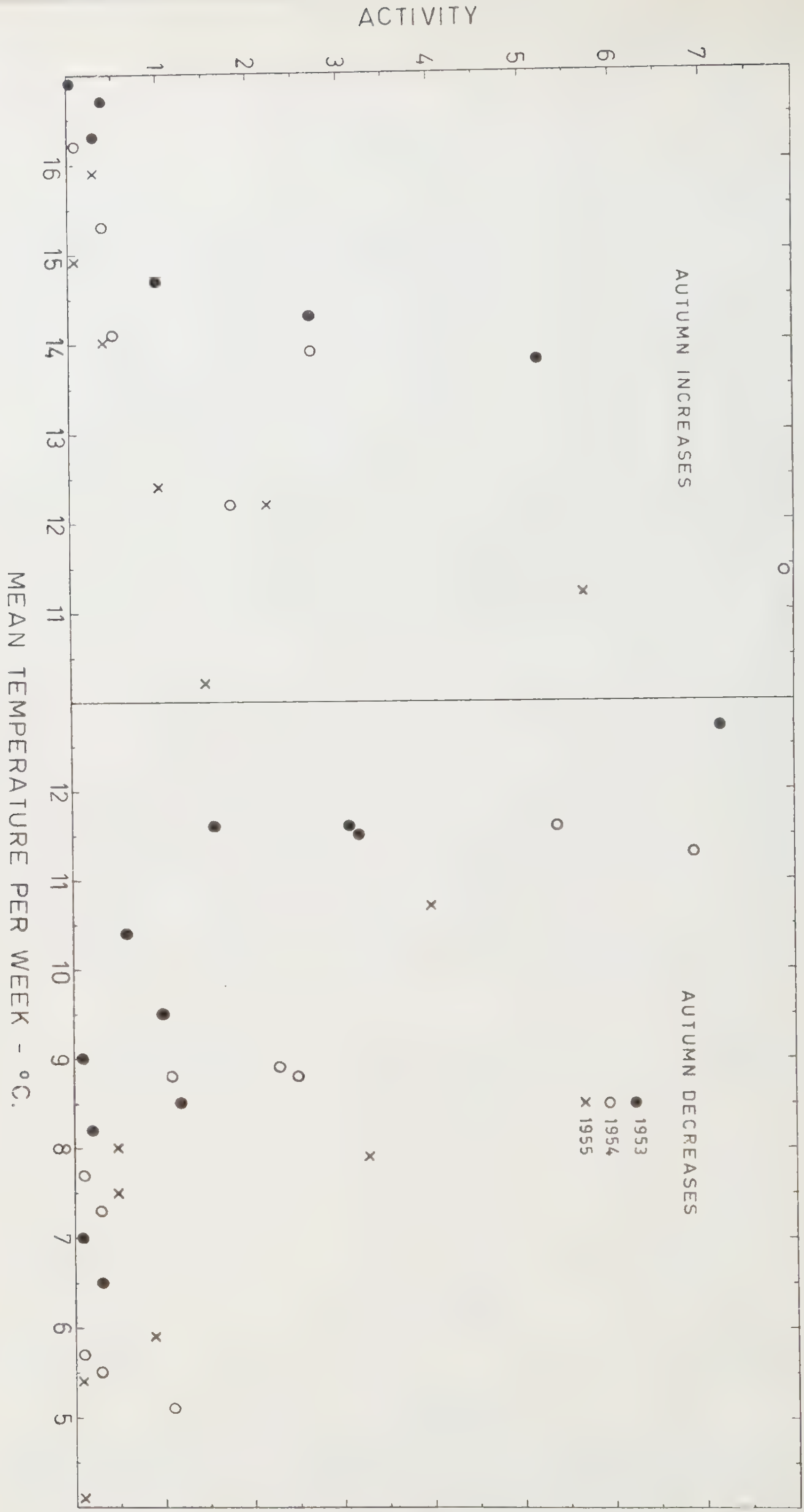

Fig. 16. Activity of *C. frisius* vs. mean temperature per week during successive weeks in which increases or decreases in the number of animals trapped were observed (c.f. figs. 6–8). Activity = number of animals trapped as % of the yearly total.

ever, the correlation of activity with temperature was much larger in this case. Partial rank correlations applied to the data in the two cases in which a significant relation to rainfall was found did not alter the differences between the coefficients for temperature and precipitation. These two relationships between activity and rainfall probably cannot be ascribed to congruity between temperature and rainfall.

Figures in table 1 certainly warrant the conclusion that activity changes of *C. frisius* at certain times of year bear very definite association with simultaneous variations in temperature. The way in which activity of this species is related to temperature is shown in figs. 15 and 16. In these graphs, the numbers of animals trapped, expressed as percentages of the total catch for the year in question, are considered as indices of activity and are plotted against the mean temperature per week for successive weeks during which increases or decreases in the number of animals trapped were observed (see figs. 6–8). Figs. 15 and 16 are not concerned with fluctuations in numbers which occurred during periods of maximum activity. The data, generally, represent curved functions which consist of two parts, one steeply sloping and the other tending to the horizontal. These diagrams confirm the relations suggested by figs. 13 and 14 and table 1. Increases and decreases in activity of *C. frisius* are apparently initiated when the mean temperature, in the field, reaches a certain value, this value depending on the time of year and whether activity is at a maximum or a minimum. When this temperature is reached, activity either increases or decreases very rapidly over a narrow range of temperature until a maximum or a minimum is attained and this activity level is then maintained until a critical temperature for either decrease or increase in activity is experienced again. These critical temperatures apparently vary slightly from year to year as well as with the time of year. Spring increases in activity were initiated fairly constantly at a temperature of about 6.5°. Decreases in spring over the three years began, however, between temperatures of 9° and about 11.5°. Autumn increases began at temperatures of 13° to 15° and decreases in autumn at temperatures between 10° and 13°. As was suggested in connection with Figs. 13 and 14, there may be a difference between the influence of rising temperature and the influence of falling temperature on activity. Increases in activity during autumn, when the mean temperature per week is falling, are initiated at higher temperature than increases in activity which occur in spring when the environmental temperature is rising. Also, maximum activity is attained at lower temperatures (9°–11.5°) in spring than in autumn (11°–14°). The fact that the critical temperature at which changes in activity begin is variable from year to year may be an indication that the relation between temperature and activity is in-

direct. Other factors may be influential in activity increases and decreases.

Besides these cyclic changes in activity, irregular fluctuations also occur within periods of the yearly cycle when activity is at a maximum (figs. 4 to 8). Some of these variations are probably random but it is also possible that they too bear some relation to concomitant alterations in temperature or rainfall. No correlations could be found, however, between activity changes at these times and either temperature or rainfall.

VI. SUMMARY AND CONCLUSIONS

The distributions and seasonal activities of three species of diplopods, *Polydesmus denticulatus* C. Koch, *Brachyiulus littoralis* (Verh.), and *Cylindroiulus frisius* (Verh.), have been examined on the basis of the number of animals trapped in different biotopes in an area of sand dunes.

P. denticulatus and *B. littoralis* have nearly identical distributions in the experimental terrain. Both are closely restricted to woods with rich and varied undergrowth or to areas thickly overgrown with grass. *C. frisius* occurs in all of the biotopes sampled in the experimental area. The greatest numbers of animals of this species generally occur in woods or in surrounding grass land but the habitat of the species extends also, although less frequently, to more exposed terrain, sometimes to almost bare sand.

All three species become active in March but the lengths of time during which they remain more or less continuously active vary. *C. frisius* is active until the end of December and *P. denticulatus* until November or December. *B. littoralis* shows very little activity between September and March.

There are also more or less clearly defined periods during the year when the species are exceptionally active. *C. frisius* has two such periods per year while *P. denticulutas* and *B. littoralis* characteristically exhibit only one, less distinct, period of maximum activity. The occurrence of these maxima in activity is related to associations of temperature and rainfall in which temperature is apparently the more influential factor. Other factors, however, are probably primarily responsible for the incidence of activity maxima.

Temperature may also be influential in the initiation of changes in activity during the year. Regularly occurring increases and decreases in activity of *C. frisius* were found to be clearly related to contemporary changes in environmental temperature but seldom to rainfall. These relations were not established for *P. denticulatus* and *B. littoralis*. Cyclic changes in activity of *C. frisius* are apparently initiated when the mean

temperature per week, in the field, reaches a certain value depending on the time of year, i.e., whether temperature is rising or falling, and whether activity is at a minimum or a maximum. The fact that this critical temperature is somewhat variable from year to year may be a further indication that factors other than temperature are also influential in determining activity of the species in the field.

No correlations were found in any of the three species between fluctuations in activity, during periods of maximum activity, and either temperature or rainfall.

VII. ACKNOWLEDGMENTS

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ON THE THEORIES OF HOST-PARASITE INTERACTIONS[1]

by

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During the last century HOWARD (1897) pointed out the significance of insect parasites in the natural regulation of their hosts. In the first quarter of this century HOWARD's opinion has been corroborated by the success of several biological control operations (CLAUSEN, 1951). On the other hand the introduction of parasites does not always succeed in reducing host density to a level low enough to eliminate economic damage. Moreover, in forests where insects, normally living at a low density level, are liable to increase to infestation proportions, indigenous parasites are sometimes found to play only a small part in control at the higher densities. In such cases parasites do not appear able either to prevent infestation in the first place, or to cause significant reduction in host numbers once the outbreak has taken place.

Hence, the part played by parasites in the biological or natural control of their hosts is not always evident. There seem to be certain conditions under which their regulatory capacity is limited. On the whole, however, it is generally assumed that parasites are important controllers of host numbers (HALDANE, 1953) though it must be admitted that this assumption is but poorly supported by reliable field work. In my opinion the most logical support may be found in the clear reasoning of NICHOLSON in his theory of host-parasite interaction, the premises of which are still to be proven.

Two important theories of host-parasite interaction have been stated, those of THOMPSON (1922-24) and of NICHOLSON (1933). The latter has been formulated mathematically by NICHOLSON and BAILY (1935).

[1] Paper discussed at the Congress of I.U.F.R.O., Oxford 1956, with some modifications added.

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THOMPSON's theory makes the initial assumption of a parasite-host relationship within which both host and parasite lay a fixed number of eggs (power of increase h and s, respectively). Starting from definite initial host and parasite densities (n and p, respectively) their numbers (G_t and P_t) after t successive generations may be expressed arithmetically. Assuming h and $s > 1$, both the host and parasite have increased in numbers after t generations. When $G_t = P_t$, the parasite will kill all the hosts. This is so when

$$t = \frac{\log \frac{na - n + pa}{na}}{\log a}, \text{ where } a = \frac{s}{h}$$

In this function t has a real value only when $na + pa > n$, and therefore when $p/n > \frac{h-s}{s}$. In other words the host will be eliminated (the percentage parasitism will increase) upon this supposition. If $p/n < \frac{h-s}{s}$ then the host and parasite will multiply in geometric progression to infinity, and the percentage parasitism will go on decreasing. THOMPSON mainly applies his theory to phenomena observed in biological control operations, and with certain reservations, it may be used to explain both the successes and the failures of this method as a means of control. Biological control, however, never results in complete elimination of the host. Once the parasite is established the host density is reduced to a level where economic damage no longer occurs, and at this level host and parasite live together in the same habitat, and their populations are in a state of fluctuating balance. THOMPSON's theory does not provide any form of a state of balance. A theoretical 'steady state', where the parasite kills as many hosts as needed to maintain the population of host and parasite unchanged, is only possible when $p/n = \frac{h-s}{s}$ and, in addition, the number of eggs laid per parasite individual is extremely small ($s = 1$) (neglecting the sex for the sake of simplification). Such a situation may be rejected as impossible because there are no parasites known with so low a fecundity. In addition, the existence of a host attended by a single specific parasite and uninfluenced by any other mortality factor, as assumed by THOMPSON, is very unlikely to occur in nature. The system will always be more complex and thus more applicable to phenomena actually observed under field conditions.

In such a relatively complicated system a steady state can exist in theory when the mortality factors account for a constant percentage

of the populations. In nature, however, the influence of mortality factors changes from generation to generation, and it will be interesting, therefore, to consider what happens under such conditions. In these considerations we must differentiate between mortality factors affecting either the host or the parasite (specific factors), and those affecting the host and parasite indiscriminately (non-specific factors). Factors of the latter type normally eliminate parasitized and non-parasitized hosts to the same relative amount.

When a specific mortality factor kills a constant percentage of hosts, the number of eggs laid by the parasite must still be 1 for the steady state to exist. It appears from the example given below, that such an equilibrium is very unstable, for when the mortality factor changes its influence on the population once (i.e. on 1 generation), the steady state is disturbed. The result is either extermination or, alternatively, infinite increase of the host, because in the first case $\frac{p}{n} > \frac{h-s}{s}$ and in the second $\frac{p}{n} < \frac{h-s}{s}$ (cf p. 135). In both cases the parasite will be incapable of increasing its numbers.

E.g. n = 40, p = 10, h = 5 and s = 1, $\frac{p}{n} = \frac{h^1-s}{s}$ *)

With specific mortality operating at 75%, and changing once to 80% (a) and 70% (b):

Steady state: hosts, 40 × 5 = 200; survival 25% = 50
parasites, 10 × 1 = 10 –
40 host
10 parasite

(a) extermination of the host

40 × 5 = 200, survival 20% = 40
10 × 1 = 10 –
30

30 × 5 = 150, survival 25% = 37,5
10 × 1 = 10 –
27.5, because $\frac{p}{n} = \frac{1}{3} > \frac{h^1-1}{1} = \frac{1}{4}$

(b) infinite increase of the host

40 × 5 = 200, survival 30% = 60
10 × 1 = 10 –
50

50 × 5 = 250, survival 25% = 62.5
10 × 1 = 10 –
52.5, because $\frac{p}{n} = \frac{1}{5} < \frac{h^1-s}{s} = \frac{1}{4}$

* Virtually through the operation of the mortality factor the host's power of increase is reduced to 25% of its original value. Instead of 5 (=h) only 1.25 (=h^1) offspring are produced per host individual.

The defect of the theory is also clear when a non–specific mortality factor operates. When this factor kills a constant percentage of parasitized and non-parasitized hosts, the number of eggs laid by the parasite must be greater than 1 for the steady state to exist. From the number of hosts parasitized by one parasite individual 1 must survive, the surplus being destroyed by the mortality factor. However, such an equilibrium is unstable as well, for when the factor changes its influence once, the steady densities will be higher than the original when mortality is reduced and lowered when mortality increases. In contrast with the foregoing example new steady densities will be maintained because the proportion p/n does not change (cf p. 135).

E.g. $n = 40$, $p = 10$, $h = 25$, $s = 20$, $\frac{p}{n} = \frac{h-s}{s}$

With non-specific mortality operating at 95%, and changing once to 80% (a) and 99% (b):

Steady state: 40 × 25 = 1000, hosts
10 × 20 = 200, parasites

800 ; 5% survival = 40, × 25 = 1000
200 ; 5% survival = 10, × 20 = 200

800
200

(a) mortality reduced
800 ; 20% survival = 160, × 25 = 4000
200 ; 20% survival = 40, × 20 = 800

3200 ; 5% survival = 160
800 ; 5% survival = 40

(b) mortality increased
800 ; 1% survival = 8, × 25 = 200
200 ; 1% survival = 2, × 20 = 40

160 ; 5% survival = 8
40 ; 5% survival = 2

Under field conditions we may expect that the percentage killed by a mortality factor changes from generation to generation and fluctuates around a mean value. If this value is such, that the condition for balance, $\frac{p}{n} = \frac{h-s}{s}$, is satisfied, and each parasite leaves one offspring in average we might logically assume that the parasite and host populations would be held in a state of fluctuating balance. Arithmetical consideration, however, lead us to the conclusion that in such a situation the parasite is unable to keep the population of the host in balance.

When a fluctuating specific mortality factor operates the result will be the same as with a specific factor changing its influence once (cf. example 1): either extermination or infinite increase of the host. The

result is depending on the type of the first disturbance of the steady state. When the mortality is reduced and, consequently, the host density is increased, so that $\frac{p}{n} < \frac{h-s}{s}$, the host will go on increasing. This increase can only be stopped by a high mortality, which reduces the host density to a level lower than the steady density. Then $\frac{p}{n} > \frac{h-s}{s}$, and the host will go on decreasing in numbers, unless by a considerable fall in mortality the host density again rises to a level higher than the steady density. This return to intermediate densities may accidentely occur by a random fall or rise in mortality when host density has not yet reached a too extreme value. Long term fluctuations, however, can only occur when the mortality factor is density dependent. In this case the population density is regulated by the mortality factor and the parasite has no regulating effect.

When a fluctuating non-specific mortality factor operates the parasite likewise is unable to keep the population in balance. When the mortality fluctuates around a mean value the population always decreases.

E.g. $n = 40$, $p = 10$, $h = 25$, $s = 20$, $\frac{p}{n} = \frac{h-s}{s}$

With non-specific mortality operating at 95% in average, and fluctuating at 92% 97%, 93% and 98% in successive generations:
Steady state, see foregoing example

800 hosts ; 8% survival	= 64 , × 25 =	1600
200 parasites; 8% survival	= 16 , × 20 =	320
		1280 hosts
1280 ; 3% survival	= 38.4, × 25 =	960
320 ; 3% survival	= 9.6, × 20 =	192
		768 hosts
768 ; 7% survival	= 53.8, × 25 =	1345
192 ; 7% survival	= 13.4, × 20 =	268
		1077 hosts
1077 ; 2% survival	= 21.5, × 25 =	538
268 ; 2% survival	= 5.4, × 20 =	108
		430 hosts

Because the mortality factor affects the host and parasite indiscriminately the proportion $\frac{p}{n}$ and, consequently, the percentage of parasitism do not change. In the above example the number of non-parasitized hosts can be calculated in each

generation as 0.80 times its initial number. Therefore, in the steady state the numbers of the host surviving the mortality factor and the parasite during two generations are as follows:

$$800 \times (\underbrace{0.05}_{\text{fraction surviving the mortality factor}} \times \underbrace{25}_{\text{power of increase}} \times \underbrace{0.8}_{\text{fraction surviving the parasite}}) \times (0.05 \times 25 \times 0.8) = 800$$

When the mortality factor fluctuates around the mean value of 0.95 (say 0.92 and 0.98 in two successive generations) the numbers of the host run as follows:

$$800 \times (0.08 \times 25 \times 0.8) \times (0.02 \times 25 \times 0.8) = 512$$

Because $0.05 \times 0.05 > 0.08 \times 0.02$, or, in general $a^2 > (a-p)\,(a+p)$, the host density always decreases.

Thus THOMPSON's theory does not in fact provide a basis for a host-parasite system in fluctuating balance, owing to the assumption that the number of eggs laid by a parasite is unrelated to host density. If this assumption is made in a parasite-host system within which specific or non-specific mortality factors are operating, disturbance of the steady state leads to infinite increase of the host or to extermination. Moreover, it seems highly improbable that, at very low host densities, a parasite individual would be capable of finding the hosts necessary for deposition of all its eggs. We might expect this to occur only at really high host densities (p. 141).

NICHOLSON's theory starts with the assumption that only the host produces a fixed number of progeny, the number of eggs laid by the parasite being dependent on host density. NICHOLSON further claims that parasites search at random and thus the probability of finding a host is proportional to host density.

The capacity of a parasite to find its host is referred to as the area of discovery, and may be defined as the area effectively searched during the lifetime. It is the area within which all hosts have been found and parasitized. The number of hosts parasitized is high when host density is high and low when the density is low. The searching capacity, and hence the area of discovery of a parasite, is dependent on the keenness of its senses and its efficiency in capture. Thus an active parasite with keen senses will find more hosts of a definite species and will have a larger area of discovery.

The fraction of hosts parasitized (named the area covered) by a certain parasite is independent of host density. Though this fraction increases when the number of parasites increases, the rise is not proportional to the parasite density because the areas traversed by different individuals overlap more and more as parasite density increases. Thus, there is an increasing probability for a parasite individual to

find a host already parasitized with rising parasite density and those hosts will be avoided by selective parasites or parasitized again by non-selective or superparasites. The relationship between fraction parasitized (= area covered = percentage parasitism) and parasite density (expressed as the area traversed by the total parasite population) is an e-function, the graph of which is named the competition curve (see NICHOLSON, 1933). When values for the area of discovery of the parasite and the power of increase of the host are known, a steady state for the insects can be calculated. As already mentioned above such a simple example is unlikely to exist in nature but it is possible to introduce all kinds of specific and non-specific mortality factors into it. Such introductions change the steady densities but otherwise will not disturb the state of stationary balance. Under field conditions, as above mentioned, a stationary balance cannot occur because the mortality factors will never be constant, and therefore this balance, assuming that it did once exist, is immediately upset. However, in a Nicholson system the reaction of the parasite to the disturbance is basically different from its behaviour in a Thompson system. When the change in influence of the mortality factor results in an increase in host density, the parasite's chance to find a host is increased and consequently more eggs are laid; this leads to a reproduction excess and an increase in parasite density in the next generation (delayed density dependent: VARLEY 1947). In this generation a greater fraction of hosts is eliminated and this is repeated in subsequent generations until the host's mortality exceeds its reproduction. Due to the delay in effect the parasite is still very numerous even though the host has decreased in numbers and, thus, host density falls far below the steady density which, in turn, causes a greater reduction in parasite numbers. The host will then recover again, but, due to the scarcity of the parasite, will reach much higher numbers than in the previous oscillation. In fact, the delay in the effect of the parasite causes oscillations with increasing amplitude. Thus NICHOLSON's theory, too, fails to provide a basis for a self-regulating system. In theory an interaction with increased oscillation always leads to a peak, followed by a crash to a very low population density. NICHOLSON claims that when such a situation has been reached the densities are maintained much below their steady values, the animals being distributed in small groups. However, under field conditions, there are many host-parasite systems not characterized by these phenomena.

There is very little field evidence to support NICHOLSON's theory. VARLEY (1947) has shown that the fecundity of the chalcid *Eurytoma curta*, a parasite of the gall fly *Urophora jeceana*, was reduced by a fall in host density. Estimates of the area of discovery of the parasite for

two years were 0.31 and 0.19 sq. m. but the difference is not significant, and moreover, the latter value may show inhibition of the parasite by cold weather.

All further evidence is from experimental work. BURNETT (1951), DE BACH AND SMITH (1941), and FLANDERS (1935) have shown in experimental populations that the number of hosts parasitized per parasite increased with increase in host density. FLANDERS (l.c.) concluded from his work with the egg parasite *Trichogramma* that increase in fecundity reaches a maximum at a definite host density owing to the limited egg-laying capacity of the parasite. Thus over a part of the range of host densities NICHOLSON's theory applies. When host density is very high the influence of the parasite may be limited by egg supply, and at this density level THOMPSON's theory holds. This has already been pointed out by VARLEY (1947).

Egg production in Hymenoptera has been studied by FLANDERS (1942). He showed that in some species production of ripe eggs continues throughout the life of the parasite as long as there are hosts enough for oviposition. When oviposition is inhibited oogenesis may continue but ovulation ceases, and the ripe eggs in the ovarioles are resorbed. Some ripe eggs, however, are always present and, as in species which store ripe eggs in the uterus, oviposition may take place at any time hosts are offered. These mechanisms may be considered as adaptations for maintaining the reproductive capacity when environmental conditions are unfavourable for oviposition. The results from FLANDER's work provide a good argument in favour of NICHOLSON's theory, for in some Hymenoptera the number of ripe eggs produced appears to be highly dependent on host density so that their fecundity cannot be constant. In other species a definite number of eggs is produced a.o. *Trichogramma* (FLANDERS) and, probably, also in many Tachinidae. In these species we may expect that NICHOLSON's theory will hold over a definite part of the range of host densities. When host density is very high the parasite will be limited by egg supply and under such conditions THOMPSON's theory will be valid.

According to the above arguments we may conclude that NICHOLSON and THOMPSON's theories are not antagonistic. They are both applicable but at different densities of the host.

It has already been pointed out that neither theory provides a sound basis for a self-regulating host-parasite complex. NICHOLSON, however, offers a better starting point than does THOMPSON. In the former any disturbance of the steady state by a changing specific or non-specific mortality factor included in the system gives rise to regular oscillations about the steady state. In the latter every disturbance of the steady state by a specific or non-specific factor leads to unlimited increase or

to extermination. Regular oscillations, inherent in NICHOLSON's theory of parasite–host relationships, cannot exist under actual field conditions because of the changing environmental factors working within the system. This does not mean that NICHOLSON's assumptions cannot be realized in nature. The main objection which may be levelled against his theory is the ever-increasing amplitude of the oscillations, which has never been observed in nature. DE BACH and SMITH (1941) have shown experimentally that this effect can, in fact, occur in a host-parasite relationship, but the conditions under which their experiments were carried out were so artificial that the results should be regarded with reservation.

In the literature several mechanisms with damping effect have been mentioned on purely theoretical grounds. NICHOLSON himself suggests as an example one parasite with two or more hosts, one of which is regulated by the parasite and the others by other factors. The density of the parasite then is, in part, determined by its other host(s) and consequently the violence of its effect on the regulated host is reduced. This leads to oscillations with decreasing amplitude. VARLEY (1947) claims that the oscillations may be damped if some of the hosts are less available to the parasite than others, and this may be so due to the irregular distribution of the host.

DE BACH and SMITH have pointed out that the oscillations may be damped by a strongly density dependent reproduction. However, they realize... 'that where an enemy really had a decided depressing influence on the population density of its host, the point in host density at which the latter limits its own reproductive rate is not ordinarily attained'. Under experimental conditions this point in density may be reached, as shown by UTIDA (1950), in populations of *Callosobruchus chinensis* and its larval parasite *Heterospilus prosopidis*. Here the host is prevented from reaching extremely high densities by its own decreasing fecundity and fertility.

The mechanism of damping was thoroughly investigated by the late L. TINBERGEN[1]. Very briefly he reviewed his ideas in a lecture published in 1955 and in which he considered three ways in which damped oscillations may be introduced into a NICHOLSON-system.

1. That given by NICHOLSON himself and which has already been mentioned. In this example the density of the host not regulated by the parasite is independent of the activity of the parasite and remains constant. In nature such a situation can not be, and we must interpret NICHOLSON to mean that fluctuation in host numbers is not caused by the parasite whose influence is counteracted by the regulating mechan-

[1] His conclusions are summarized here at the request of the discussion group on population – dynamics of the 24th Section of I.U.F.R.O.

ism of the host itself. However, when there are high densities of the parasite owing to large numbers of the regulated host, it is very unlikely that the regulating mechanism of the second host will be able to counterbalance a high mortality.

2. *Density dependent reproduction of the host.* TINBERGEN has shown arithmetically that a strongly inverse density dependent reproduction of the host results in damped oscillations in any host-parasite system. Therefore fecundity and fertility must fall greatly at density levels normally occurring in natural populations. TINBERGEN, as did DE BACH and SMITH (1941), realized that density dependent reduction of fecundity at these relatively low densities is very unlikely to exist in actual fact. It has been shown by MORS (1942) that at the very high densities present during an infestation of the Nun Moth, *Lymantria monacha*, both the fecundity of the female and the power of copulation of the male were reduced.

3. *Density dependent mortality of the host.* In theory density dependent mortality, as in the case of reproduction, proves to have a damping effect, provided that the factor involved plays an important part in mortality at intermediate densities of the host. This condition is much more likely to exist in nature. Having once established this, TINBERGEN proceeded to combine his theory with facts gained in his studies on mortality in caterpillar populations caused by titmice. He measured percent predation of several caterpillar species at various densities over a number of successive years. At lower hos densities mortality proves to be density dependent in the sense that SMITH uses this term (1935). At higher densities mortality appeared, however, to be inversely density dependent in the sense given by SOLOMON (1949). Thus the relation between prey density and percent predation will have the appearance of an optimum curve.

The density dependent effect is due to the development of a preference by the titmice for prey species at intermediate densities (the biological significance being an increase of efficiency in foraging). At low densities of the prey this preference does not develop because, at such levels of population, the rarity of the prey makes a specific search an inefficient method.

TINBERGEN combined the empirically determined optimum curve of particular prey species (*Panolis* and *Acantholyda*) with a NICHOLSON-parasite – host complex and he concluded that the density dependent effect at lower densities causes damped oscillations.

Hence, in theory, a combination of bird and parasite predators on a common host provides a very good example of a self-regulating system. It is probable that the role given to birds in the above example may be taken up by other factors in habitats where birds are rare or absent.

TINBERGEN's studies have stressed the great value of NICHOLSON's theory. The conclusions he reached should act as a strong stimulus to further investigation into the interaction of host and parasite, and to test the correctness of NICHOLSON's basic assumptions under field conditions. VARLEY's work on *Urophora jaceana* and its parasites shows clearly how best to set out on field work of this type, in spite of MILNE's (1957) criticism on its statistical methods and mathematical considerations. This criticism is partly right, but in fact does very little derogation to the general line of thought and the way of planning population dynamic field work. It is essential to obtain host and parasite density and percentages of parasitism over a period of generations. Forests are particularly suited to this type of investigations, owing to their comparative constancy of condition from year to year, and to their usually simple homogeneous plant composition. Therefore forest entomologist's seem to have a special task in this field of ecology.

SUMMARY

The theories of host-parasite interactions of THOMPSON and NICHOLSON are discussed. The first theory makes the initial assumption that both host and parasite lay a fixed number of eggs. NICHOLSON, on the other hand, assumes that only the host produces a fixed number of progeny, the number of eggs laid by the parasite being dependent on host density.

Further, both theories assume that the host is attended by a single specific parasite (THOMPSON and NICHOLSON) or more, and also non-specific (polyphagous) parasites (NICHOLSON). In nature, however, the system will always be more complex and both host and parasite will be influenced by other mortality factors. Moreover, the effect of these factors changes from generation to generation.

When fluctuating mortality factors operate in a THOMPSON parasite-host system the inevitable disturbance of the steady state leads to infinite increase of the host or to extermination, the result being dependent on the properties of the mortality factor. This is illustrated in the text with some arithmetical examples. Thus, THOMPSON's theory does not in fact provide a basis for a host-parasite system in fluctuating balance, owing to the initial assumption that the number of eggs laid by the parasite is fixed and unrelated to host density.

When fluctuating mortality factors are included in a NICHOLSON parasite-host system the reaction of the parasite causes oscillations with increasing amplitude. This leads to a peak, followed by a crash to a very low density. However, under field conditions, there are many host-parasite systems not characterized by this phenomenon.

In the literature several mechanisms damping the increasing amplitude inherent to a NICHOLSON system have been mentioned on theoretical grounds. The most interesting is that given by L. TINBERGEN. He measured percent predation caused by titmice in caterpillar populations at various densities over a number of successive years. In the range from low to intermediate densities of the prey mortality proved to be density dependent. When this empirically determined effect is combined with an arithmetical NICHOLSON host-parasite system it results in damped oscillations.

Hence, in theory, a combination of bird and parasite predators on a common host provides a very good example of a self-regulating system.

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A HISTO- AND CYTOLOGICAL STUDY ON THE PITUITARY OF THE STICKLEBACK *GASTEROSTEUS ACULEATUS* L. FORMA *TRACHURA* CUV. PARTLY BASED ON A NEW FIXATION PROCEDURE AFTER FREEZE DRYING

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CONTENTS

I. GENERAL INTRODUCTION

Modern histological and endocrinological investigations have shown the existence of an interesting cellular basis for the endocrinological function of the pituitary: the production of several hormones, such as follicle stimulating hormone (FSH), luteinizing hormone (LH), and thyrotrophin (TSH) could be localized in the pars anterior of the pituitary. Moreover, for every hormone tinctorially different cell types, which appeared to have a definite spatial localization, could be indicated. The experiments have been done with mammals, mainly the rat (Catchpole, 1949–'50; Halmi, 1950, 1951, 1952a, 1952b; Hildebrand, Rennels and Finerty, 1957; Purves and Griesbach, 1951a, 1951b, 1951c, 1954, 1956; Rennels, 1957; Wilson and Ezrin, 1952).

Successful experiments of this kind seem to have been done only on a limited scale with animals of the group of Teleost fishes. According to the nowadays generally accepted opinion in Teleostei both thyrotrophin and gonadotrophin (there is only scarce evidence for the existence of FSH, the gonadotrophin in fishes should be only LH) are produced in the transitional lobe of the pituitary (Pickford and Atz, 1957). Up till now the only investigators, who seem to have been able to stain gonadotrophs and thyrotrophs differentially in a teleost's pituitary are Atz (1953), working with the Characin (*Astyanax mexicanus* (Filippi)) and Barrington and Matty (1955), working with the Minnow (*Phoxinus phoxinus* L.).

Though it is not the aim of this study to relate the production of the different hormones to more or less sharply defined cells, a description of the cell types which can be found in the pituitary of the teleost *Gasterosteus aculeatus* L. forma *trachura* Cuv., the Three spined Stickleback, will possibly be of value for such a future correlation and for an endocrinological and ethological understanding of this animal.

The Plated Stickleback (*Gasterosteus aculeatus* forma *trachura*) is the object of these investigations, whereas BOCK (1928) has given a description of the pituitary of *Gasterosteus aculeatus* L. forma *leiura* Cuv. (= forma *gymnura* Cuv.), the Half plated Stickleback. As far as the present author can judge from the description of BOCK and from limited investigations of forma *leiura*, the histology of these pituitaries does not differ greatly. However, in the past 30 years some new histological and histochemical staining methods have been developed which could throw a new light on the hormonal organisation of this fish. Moreover, some additional structural peculiarities of this pituitary can be given.

Some reasons why the Three-spined Stickleback is chosen as experimental object are:

1. By the work of TINBERGEN and TER PELKWIJK (1937), VAN IERSEL (1953), MORRIS (1958), and SEVENSTER (1958) many particulars, especially about the sexual behaviour of the Stickleback, have become known.
2. It tolerates experimental interferences extremely well: BOCK (1930, cf. VON BUDDENBROCK, 1950) was able to keep Sticklebacks alive for some months after castration; whereas the present author succeeded in keeping this fish alive for 39 days after castration.
3. During its migration to or from the sea the animal is easily obtainable in large amounts.

It is our intention to investigate in the future whether a correlation exists between different hormones and different cell types, between the hormonal organization of the pituitary of the Stickleback and its sexual behaviour. Here, as a preliminary to the above mentioned subjects, a histo- and cytological description of the pituitary of the Stickleback will be given, based on staining results obtained from pituitaries of adult specimens, which were killed mainly in winter and spring.

II. MATERIAL AND METHODS

For the histological and histochemical description of the pituitary of *Gasterosteus aculeatus* L. the forma *trachura* Cuv., the Plated Stickleback, was used exclusively. The fishes were caught in the "Noordhollands Kanaal" at Den Helder (brackish water), in the "Hollands Diep" (brackish water) and in the "Oude Maas" at 's Heerjansdam (fresh water) at different times during their migration to the sea. After transport to the laboratory they were kept in aquaria with fresh water. Adult animals, measuring more than 4 cm in length, were exclusively used. The animals were killed by cutting off the head. The bones of the skull floor were removed and the brain with the adhering hypophysis lifted out. In the case of liquid fixation the pituitary was fixed together

with the brain, whereas the brain was removed in the case of freeze drying. These two procedures will be treated separately.

Liquid fixation. A large number of pituitaries was fixed in liquid fixatives, such as formol 4%, formol sublimate (1 part formol 40% + 9 parts watery saturated sublimate solution), Zenker acetic and Zenker formol. Upgrading was carried out through alcohol, methylbenzoate and benzene, embedding in paraffin, mp. 58°C. Section thickness was 5 μ, if not stated otherwise. Mostly longitudinal sections were cut. Hot xylene was used for deparaffinizing, after which the slides were graded down to water via alcohol. If necessary, sublimate was removed in 0.5% I_2 in alcohol 70%, followed by 0.25% sodiumthiosulfate.

Freeze drying. Originally no satisfactory results were obtained with pituitaries of the Stickleback using the technique of freeze drying followed by absolute alcohol fixation. As it seemed probable that the bad results were due to inadaequate fixation a new method of fixation was worked out (see chapter III). The brains of about 20 animals were used for this fixation investigation. Part of these could also be used to obtain the histological data described in chapter IV. They were trimmed under a binocular lens to such dimensions that the blocks of tissue did not exceed 1 mm in thickness. Quenching was carried out in a CO_2–acetone mixture, if not stated otherwise. Freeze drying was performed in an Edwards TD 2 tissue dryer, the drying time being 42 hours at a temperature of -45°C. After this the specimens were fixed in formolgas, embedded in paraffin and sectioned longitudinally at 5 μ thickness.

The following staining methods were applied:

1. acid hemalum Mayer (or Harris' hematoxylin) —eosin (watery or alcoholic); abbreviated as HE,
2. Azan (modification according to Heidenhain),
3. Mallory,
4. Gomori's chrome hematoxylin phloxin staining; abbreviated as CHP,
5. iron hematoxylin (according to Heidenhain),
6. periodic acid Schiff reaction; abbreviated as PAS,
7. PAS-acid hemalum Mayer (or Harris' hematoxylin),
8. PAS-thionin thionylchloride,
9. PAS-methylblue staining,
10. Feulgen reaction,
11. Gomori's aldehyde fuchsin staining; abbreviated as AF,
12. panoptic staining according to Pappenheim, or Giemsa and May-Grünwald staining separately,
13. acridin orange staining,
14. methylene blue staining.

Some particulars on these staining methods are given below:

Ad 2. The Azan staining in the modification according to Heidenhain was carried out as prescribed by Romeis, 1948. Azocarmine B (Dr. K. Holborn und Söhne, Leipzig) was used at 60° C, with preheating of the slides.

Ad 4. Although originally (Gomori, 1941) refixation during 12–24 hours in Bouin is prescribed, this is omitted here as no staining differences could be observed with or without refixation.

Ad 6–10 inclusive. The Schiff's solution was prepared according to Coleman, cf. Glick, 1949. Basic fuchsin of the "Amsterdamse Chinine Fabriek" and of Hopkin and Williams, Ltd., were used. Oxidation was carried out in periodic acid 0.5 % during 10 min., staining during 30 min.

Ad 8. The PAS-thionin thionylchloride stainings, a red PAS and blue Feulgen reaction, was carried out according to both prescriptions given by Van Duyn (1954 and 1956). The nuclei were always stained first. The time for optimum hydrolysis appeared to be 7 min. after formol sublimate fixation.

Ad 9. The PAS reaction was performed as usually. Though originally methyl blue is used with orange G in the same solution (Lillie, 1951) some investigators use these stains separately (Wilson and Ezrin, 1952, and Rennels, 1957). Here merely methyl blue is used in a 0.01 % solution in halve saturated picric acid. Staining time is about 15 minutes.

Ad 11. In this investigation $KMnO_4$–H_2SO_4 during 1 minute is used as oxidation method, if not stated otherwise. The staining time in the AF solution, which is prepared according to Gomori (1950) is 1 or 1.5 min.

For comparison with the results of the above mentioned method of oxidation, Lugol's solution (as in the original method according to Halmi (1952) for staining thyrotrophic hormone in the rat pituitary) during up to 4 hours, 0,5 % I_2 in alcohol 70 % during up to 6 hours and saturated watery $HgCl_2$ solution during up to 46 hours (this is included in the fixation if carried out in formol sublimate) are used. In these cases the staining times were 30 min., 30 min., and 1–3 days respectively.

Ad 12. The panoptic staining was performed as follows: staining in May-Grünwald solution during 45 min. at 37° C, prepared by dissolving 1 part May-Grünwald stock solution (Dr. K. Holborn und Söhne, Leipzig) in 8 parts primary and secondary phosphate buffer at pH 7.0, followed by staining in Giemsa solution during 40 min. at 37° C, prepared by dissolving 0.2 part Giemsa stock solution (prepared from Giemsa powder of Dr. Lamers en Dr. Indeman) in 15 parts buffer

pH 7.0. Differentiation in 0.005 % acetic acid. Mounting in a neutral mounting medium.

Ad 13. 0.01 % Acridin orange in half the usual concentration of McIlvaine's phosphate–citric acid buffer at pH's varying between 2 and 6 are used to stain for fluorescence microscopy.

Ad 14. M/2000 solutions of methylene blue in McIlvaine's phosphate citric acid buffer at pH's varying between 3,1 and 5,1 are used.

Except in the above mentioned cases of the Romanowsky type staining methods, the sections were mounted in Canadabalsam.

Whereas most sections were stained without a definite sequence of staining methods, sections of 4 pituitaries were used for staining in such a sequence that the results of a certain commonly used staining method could be compared, as far as possible, with the results of special staining methods in adjacent sections. The commonly used staining methods include HE, Azan and Mallory; the special staining methods include PAS-hemalum Mayer, PAS-thionin thionylchloride, AF, and the panoptic staining method.

The two pairs of pituitaries, each composed of one from a male, the other from a female, were fixed in formol sublimate and Zenker acetic, respectively. Every slide was provided with 3 sections of 5 μ. Out of these 4 series the 12 slides around the medial section were chosen for staining. These 4 series were stained according to the scheme:

Slide no.	Commonly used staining method	Special staining method
1	HE	
2		PAS-thionin thionylchloride
3	Mallory	
4		panoptic
5	Azan	
6		PAS-thionin thionylchloride
7	Mallory	
8		AF
9	Azan	
10		PAS-hemalum Mayer
11	HE	
12		AF

The results of these staining procedures, carried out in the above mentioned sequence, after different fixations and the results of staining methods not applied in a definite sequence, both after liquid fixations and after freeze drying, are brought together in chapter IV.

III. FORMOLGAS FIXATION OF TISSUE PIECES AND SECTIONS AFTER FREEZE DRYING

A. INTRODUCTION

The conventional way of freeze drying, that is freeze drying and fixation in absolute alcohol or acetone, gives very poor staining results with the pituitary of the Stickleback. After Azan staining the dye binding capacity is low for all three dyes, even after prolonged staining times. The PAS reaction is negative for the transitional lobe and negative or faintly positive for the pars intermedia, the PAS positive structures in the pars nervosa, however, are stainable after freeze drying and absolute alcohol fixation.

It seemed likely, that this loss of stainability of the section could be caused by a dissolution of cell substances out of the various pituitary parts. This could take place when the section is fixed and graded down to water before staining.

On the other hand freeze drying can be regarded as maintaining the cytochemical localization of cell substances very well: in a very short time the tissue is frozen and consequently dislocation by diffusion of cell substances is practically ruled out. That this diffusion can take a serious character in the technical treatment of tissues for cytochemical investigations has been shown by Bell (1956). So if it is desirable to rule out the appearance of this dislocation (and of course of dissolution) of the acidophilic and basophilic cell substances in the adenohypophysis as far as possible, freeze drying could be a good approach. For the same reason it seems favourable to leave the tissue dry at fixation after freeze drying. This can be accomplished by the use of a gaseous fixative, e.g., by taking advantage of the equilibrium:

$$\text{solid paraformaldehyde} \rightleftarrows \text{gaseous formaldehyde.}$$

Formaldehyde is an excellent fixative for glycoproteins, because it brings them into an insoluble state. In the next experiments pituitaries thus treated are compared with hypophyses, which have been fixed, after freeze drying, in the conventional way by means of absolute alcohol or acetone.

It could be argued that, if the procedure applied to the pituitary in toto (see B. pag. 154) gives valuable results, this fixation could also be applied to unfixed sections. Such experiments are described under C (pag. 160).

B. FORMOLGAS FIXATION OF FROZEN DRIED PITUITARIES IN TOTO

1. *Material, Methods and Results*

After killing the fish by decapitation, the brain with the pituitary is removed from the skull. The pituitary is removed from the brain under a binocular lens. Quenching is carried out in a CO_2-acetone mixture. Although the procedure of preparation and quenching of these small objects requires some routine, it is feasible nevertheless in a short time and thus eventual sub- and postmortem changes are reduced to a minimum. After this, freeze drying is performed in an Edwards TD 2 apparatus in the usual way. At a drying temperature of $-45°$C or slightly lower a drying time of 42 hours is more than sufficient. The dried pieces of tissue are divided into four groups in both experiments to be described. Every group is differently treated:

1. conventional fixation in absolute alcohol;
2. conventional fixation in acetone (in the second experiment only);
3. no fixation;
4. fixation in formolgas.

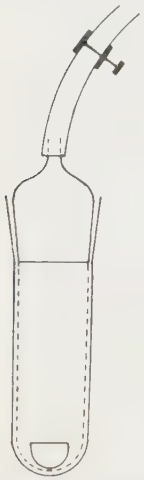


Fig. 1. Glass vessel with porcelain cup used for formolgas fixation of tissue pieces after freeze drying.

Ad 1 and 2: Samples, which are brought into absolute alcohol or acetone under vacuum (about 2 cm Hg), are upgraded through methylbenzoate and benzene to paraffin (m.p. 65°C).

Ad 3: Specimens, which are taken out of the drying head, are kept in closed glass tubes until they can be treated together with the pieces of tissue which have undergone a treatment with formolgas (see ad 4).

Ad 4: Formolgas fixation of the dried specimens is carried out in the following way. A glass vessel (8 cm in length, 2.5 cm in diameter) with a ground stopper is used. The glass tube which is connected to the stopper is provided with a piece of rubber tube which can be squeezed with a stopcock (see fig. 1). The inner surface of the vessel is coated with a layer of paraformaldehyde. This can be done in two ways:

1. The vessel is connected to another of the same kind, into which some powdered paraformaldehyde is placed. Now the two vessels are evacuated by means of a water jet pump. The vessel which contains paraformaldedyde is heated to 180–200°C on a sandbath. At this temperature the chain-polymer mixture depolymerises and gives anhydrous, gaseous formaldehyde (Fieser and

FIESER, 1950). This fills the space in both vessels. On the cold surface of the first vessel the gas polymerises to form paraformaldehyde, giving it a continuous white layer of the polymere.

2. A simpler way is to grease the inner surface of the vessel with an ordinary stopcock grease on rubber basis, then to bring in some paraformaldehyde and to rotate the vessel in order to distribute the powder smoothly over the inner surface. This method works just as well as that mentioned first.

In this investigation, however, the first method is applied. The coated vessels are kept closed by squeezing the vacuum tube. The specimen to be fixed is brought into the vessel in a small porcelain cup (see fig. 1). Before and after the specimen is brought in, the vessel is evacuated down to about 2 cm Hg by means of a waterjet pump. From the moment the rubber tube is squeezed the time of fixation is measured. At the end of fixation air is admitted through the stopcock and the sample is removed from the vessel.

The specimens which are not fixed (see ad 3) are now carried along with the fixed samples. The pituitaries are brought into the drying heads on paraffin (m.p. 65°C) which has been degassed beforehand. A high vacuum is now applied. Hereafter the samples are embedded in the usual way. Re-embedding and orientation is carried out in paraffin of the same melting point under a binocular lens.

Experiment I

Two pituitaries are fixed in absolute alcohol during 24 hours, 2 have undergone no fixation and 7 are fixed in formolgas for the following times: 20–45–90 min., 3–6–24–47 hours. This series served to determine the critical fixation time: this being the minimum fixation time necessary for maximal colour development as revealed by the PAS staining procedure. At the same time the influence of overfixation, if any, can be studied and the results obtained can be compared with those of absolute alcohol fixation and no fixation. For every fixation time a separate vessel is coated, starting from 60 mg paraformaldehyde. 5 μ thick sections are cut. Flattening is carried out on water, as it is quite unimportant to use other fluids for this purpose or to flatten dry, because all substances which should have remained soluble, dissolve later on as the preparation is graded down to water. The sections are deparaffinized in xylene, graded down through xylene-dioxane and dioxane-water mixtures to water. The following staining methods are applied:

1. PAS reaction,
2. AF staining,
3. Azan staining (modification according to Heidenhain),
4. CHP staining.

1, 3 and 4 are performed as described in Chapter II under ad 6–10, ad 2 and ad 4, respectively. AF staining is applied here exclusively after $HgCl_2$ oxidation for 46 hours. A short rinse in water is interposed between this and a 2 min. treatment in 1/3 % I_2 in alcohol 70 %, which is followed by 2 min. in 0.25 % sodium thiosulfate. After being thoroughly washed the sections are placed in the AF reagent for 1 or 2 days. Canadabalsam is used as mounting medium.

Both the Azan and CHP staining methods are carried out progressively and moreover all sections which have to be stained according to the same staining method are treated in bulk. This favours the objectivity in comparing the staining results in these two experiments.

The results which are obtained in this experiment by the different staining methods on differently fixed whole hypophyses are compiled in table I.

TABLE I

tr.l. = transitional lobe; p.ant. = pars anterior; bas. = basophilia; ac. = acidophilia

		Staining method					
	Fixation	A PAS tr.l.	B AF (bas.) tr.l.	C Azan (bas.) tr.l.	D CHP (bas.) tr.l.	E CHP (ac.) tr.l.	F CHP (ac.) p.ant.
1	Abs. Alc.	—	—	—	—	±	+
2	Abs. Alc.	—	±	±	—	—	±
3	No	—	—	—	—	—	—
4	No	—	—	—	—	—	—
5	Formolgas 20′	±	±	+	—	±	±
6	Formolgas 45′	+	+	+ +	+	+	+
7	Formolgas 90′	+ +	+ +	+ +			
8	Formolgas 3 h	+ +	+ +	+ +	+	+	+
9	Formolgas 6 h	+ +	+ +	+ +	+	+	+
10	Formolgas 24 h	+ +	+ +	+ +	+	+	+
11	Formolgas 47 h	+ +	+ +	+ +	+	+	+

The evaluation of the preparations occurs visually at the same magnification and at the same illumination. The estimations of the average intensity of the stained spots with one and the same staining method (vertical columns) are given as follows: — negative or very faintly positive; ± faintly positive; + positive; + + strongly positive.

One should not allow compare estimations which are placed on horizontal lines.

Experiment II

As the results of the various fixation times described in the first experiment could have been influenced by biological differences be-

tween the various animals, the second experiment is made in which this influence is ruled out.

After freeze drying 4 pituitaries are cut longitudinally with a razor blade. The one half of every organ is fixed in formolgas during 3 hours, the others have undergone absolute alcohol fixation, acetone fixation and no fixation (2 objects) respectively. The remaining treatments are the same as in experiment I. After cutting series of sections of every half, the sections are stained with the same methods as in the first experiment. So equally stained sections, from one and the same animal, the one fixed in formolgas, the other having undergone absolute alcohol or acetone fixation or no fixation, can be compared.

The results, obtained with the 4 pituitaries divided into two halves are compiled in table II.

TABLE II

tr.l. = transitional lobe; p.ant. = pars anterior; bas. = basophilia; ac. = acidophilia.

		Staining method				
	A PAS	B AF	C Azan (bas.)	D CHP (bas.)	E CHP (ac.)	F CHP (ac.)
Fixation	tr.l.	tr.l.	tr.l.	tr.l.	tr.l.	p.ant.
1 Formolgas	+	+ +	+ +	+	+ +	
2 No	—	±	—	—	+	
3 Formolgas	+	+ +	+ +	+	+	+
4 No	—		±	—	+	±
5 Formolgas	+ +	+ +	+ +	+	+ +	+ +
6 Abs. Alc.	±	±	+	—	—	—
7 Formolgas	+ +	+ +	+ +	+	+	
8 Acetone	—	+	+	—	+	+

The method of evaluation is the same as in table I. Here again one should only allow to compare the estimations in each of the vertical columns.

2. *Discussion*

With respect to dislocation of the easily water soluble glycoproteins and an eventual total dissolution of these substances after alcohol or acetone fixation, formolgas fixation on frozen dried pieces of tissue seems to suit the purpose of avoiding dislocation by fixing the glycoproteins in an insoluble form.

The gaseous formaldehyde is obtained at room temperature according to the equilibrium equation: paraformaldehyde $\leftrightarrows$ formaldehyde.

At room temperature the equilibrium lies far to the left, sufficient gas, however, evolves to bring the glycoproteins into an insoluble state within a reasonable time (see below). At the beginning of fixation the vessel is evacuated by a water jet pump to about 2 cm Hg in order:

1. to obtain freshly regenerated formolgas, because gaseous formaldehyde is sensitive to air oxidation (FIESER and FIESER, 1950).
2. to facilitate, by increasing the mean free path, the diffusion of the gas in the sample, to which a spongy character can be ascribed.

Formolgas proves to be able to keep the glycoproteins in the tissue (as revealed by the PAS staining, see table I, A5–A11). Sections of tissue which are adequately fixed in this way, can stay in water for a long time, without a decrease of the PAS staining intensity. It is accepted that the intensity of the staining is in proportion with the amount of cell material. From column A in table I it also appears that 90 minutes can be called the critical fixation time. In the second experiment a safety factor 2 has been used, the fixation time being 3 hours.

Not only the PAS method appears to give a maximal staining intensity after 90 minutes fixation, this is also the case with the AF staining, the anilin blue staining in the Azan procedure, the chrome hematoxylin and the phloxin staining of the CHP method (tables I and II). From this it appears that other basophilic and acidophilic protoplasmic substances are fixed as well. If the samples are not fixed (lines 3 and 4 in table I) these substances appear to be dissolved, as is revealed by the staining methods. Moreover, absolute alcohol appears to be no or an insufficient fixative for the substances stained by the methods mentioned here. Although alcohol can prevent the rehydration of frozen dried proteins (BELL, 1956), this does not appear to hold here: the proteins stay in the sections in insufficient amounts.

That dissolution or dislocation occurs is substantiated by the following experimental results:

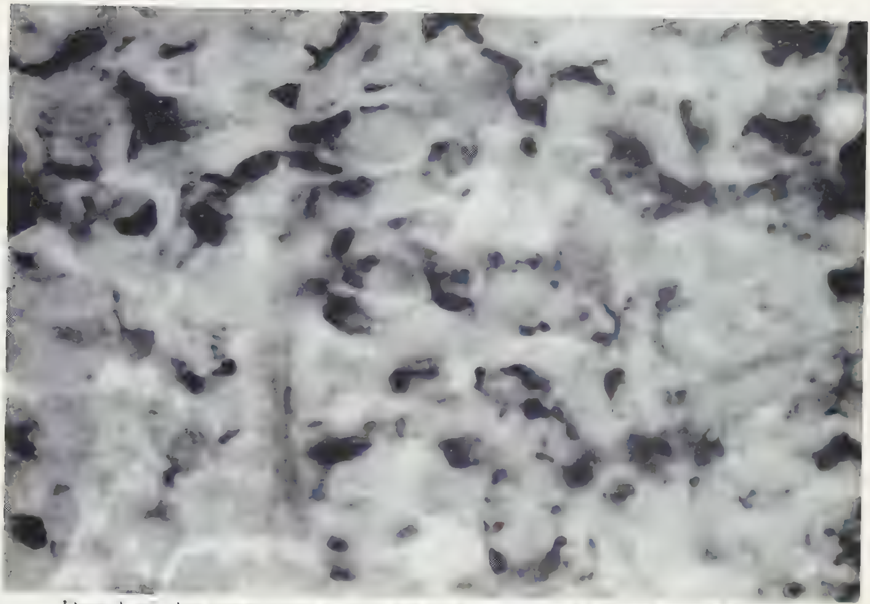
a. if a frozen dried pituitary is fixed in absolute alcohol, upgraded to paraffin, embedded in paraffin, sectioned, dry mounted on slides, deparaffinized in xylene, rinsed shortly in absolute alcohol, dried by evaporation and then fixed in formolgas (or in a 4% or 36% watery formaldehyde solution), only a faintly positive PAS staining is obtained,

b. if an unfixed piece of tissue is embedded in paraffin (immediately after freeze drying), sectioned, dry mounted on slides, deparaffinized in xylene, rinsed shortly in absolute alcohol, dried by evaporation and then fixed in formolgas, a strongly positive PAS staining is obtained.

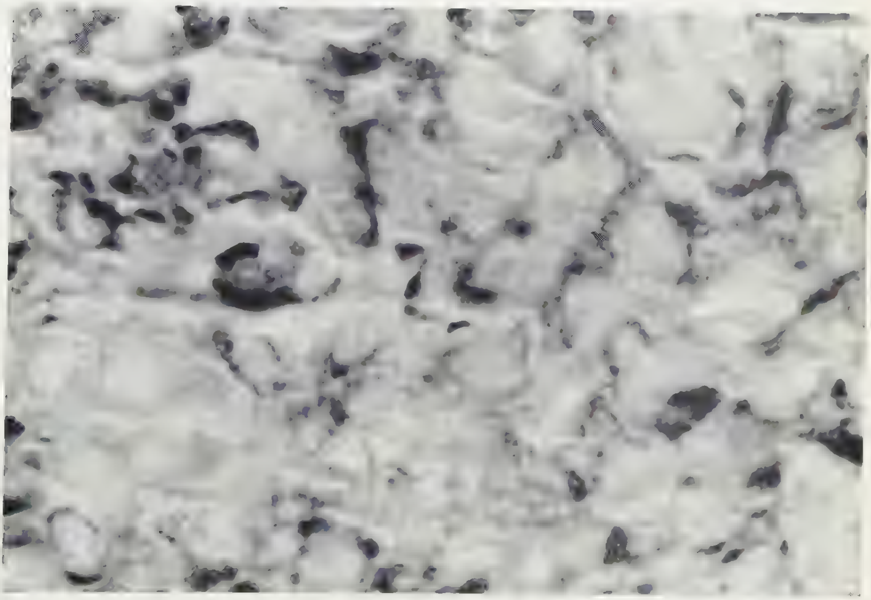
From both these experiments it can be concluded that the loss of stainable material took place during the fixation in absolute alcohol and/or during the upgrading to paraffin.

The temperature to which the samples are exposed at the stage of embedding does not seem to have a great influence on the solubility of proteins in this case (BENSLEY and HOERR, 1934, mentioned in BELL, 1956). The sections of the unfixed pieces of tissue stain badly or do

PLATE I

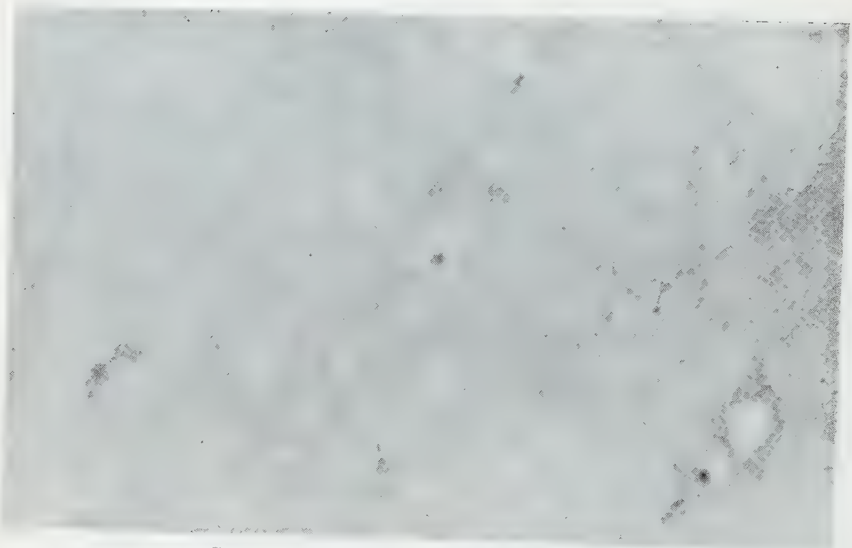


1. Detail of the transitional lobe after formol liquid fixation. PAS. × 975.



2. Same as Plate 1—1, after formolgas fixation. PAS. × 975.

PLATE II



1. Same as Plate 1—1, unfixed. PAS. × 975.

not stain at all. Anyhow a variable influence of this kind can be supposed to be ruled out as all specimens have stayed equal times in the hot paraffin.

The results of the second experiment, which affirm the results of the first, are:

1. little influence of the temperature during embedding on the cell substances investigated is observed in the unfixed pituitary halves (table II, lines 2 and 4),

2. the cell substances in the unfixed halves are dissolved out during the aftertreatment (table II, lines 2 and 4),

3. absolute alcohol is no or an insufficient fixative for the substances investigated.

Moreover, acetone also appears to be no or an insufficient fixative. In all cases fixation with formolgas during 3 hours, at an initial pressure of 2 cm Hg, appears to produce a better stainability (Table II, lines 1 and 2, 3 and 4, 5 and 6, 7 and 8, with the exception of E3 and E4, E7 and E8). It seems likely that this better stainability reflects a better preservation of the glycoproteins and of other basophilic and acidophilic substances.

The results obtained in the first experiment (column A-F in table I) cannot be explained on the basis of biological differences between the animals, as the same colour differences can appear in one and the same animal (two halves experiment), the colour differences are thus caused by the various times of fixation in formolgas. Within one series of sections such great differences as observed here, have never been found.

The cytological details which can be seen after gasfixation differ from those after formol liquid fixation. In the case of formol liquid fixation more or less sharply defined granules and more or less homogeneous spots are seen after PAS staining (see Plate I 1). After formol gasfixation the picture is principally the same, a slight difference, however, can be observed in the sharpness of the granules and the homogeneity of the spots (see Plate I 2). It seems that after formol gasfixation and PAS staining the cytological details are more distinct.

For purpose of comparison Plate II-1 shows the results of a PAS stained section of an unfixed pituitary. Absolute alcohol fixation gives almost the same picture.

C. FORMOLGAS FIXATION OF TISSUE SECTIONS AFTER FREEZE DRYING

1. *Introduction*

As mentioned on p. 153 formolgas fixation can also be applied after the frozen dried piece of tissue is embedded in paraffin, sectioned, and the sections are attached to slides and deparaffinized. These slides are placed in a cylindrical vessel, 32 mm in diameter and 135 mm in length with a ground stopper with a stopcock. The inner surface of the vessel is coated in the second of the two above described ways with a layer of solid paraformaldehyde, using 330 mg for this purpose. By means of a water jet pump a vacuum is applied before and after the slides are put in. The fixation time is measured from the second evacuation. Fixation of unfixed tissue sections can have some advantages in certain cases: after the organ is sectioned some sections can be chosen to be fixed, whereas others, e.g., next in series to the fixed ones, can stay unfixed. This working method may be advantageous in solubility experiments.

Here again, first of all the critical fixation time, as revealed by the PAS staining, is determined, after which the influence of overfixation, if any, is investigated and other staining procedures are applied.

2. *Material, methods and results*

Two pituitaries were frozen dried in the usual way. Following that, they were cut longitudinally in two parts with a razorblade. One part of each pituitary was fixed in formolgas, serving as a control. Both other parts were impregnated with paraffin in the unfixed state and were used after sectioning in an experiment in which series of fixation times (0–72 h) were employed to determine the critical fixation time and the influence of overfixation.

A technical difficulty was experienced in making the sections adhere to the slides. It appeared that in our object, containing the easily water-soluble glycoproteins, all contact with water had to be avoided; even the use of the usual albumen adhesive (chicken albumen and glycerol 1 : 1, thus containing much water) with dry mounting of the sections for the rest led to microscopically clearly visible dislocations of the substances investigated. Total omission of albumen adhesive, as is usual in dry mounting, very frequently appears to result in a swimming off of the sections. A favourable compromise between both difficulties was reached by rubbing in the slides with albumen adhesive the day before use and to dry them in the 40° C thermostat during the

night. Most of the water has evaporated out of the albumen at the time the sections are mounted. The adhering capacity is till good, while after adequate fixation no dislocations are observed.

Three methods for mounting result:

1. dry mounting on "dried" albuminized slides,
2. flattening on acetonitril on "dried" albuminized slides,
3. flattening on mercury and attaching to "dried" albuminized slides.

The second method[1] is used in the experiment which will be described next, in which the influence of fixation time on the preservation of the glycoproteins in the transitional lobe and the pars intermedia is investigated.

After fixation the slides are put in water and stained by the PAS technique. Valuation is carried out microscopically, while a PAS stained section of the control pituitary half (formolgas fixation on the piece of tissue) obtained the valuation + + for the transitional lobe and ± for the pars intermedia. The experiment is recorded in table III.

TABLE III

Fixation in formolgas in hours	0	½	1	2	4	8	16	24
PAS staining intensity in:								
transitional lobe	—	—	—	—	—	—	±	+ +
pars intermedia	±	±	±	±	±			

The influence of still longer fixation times is investigated in the next experiment (table IV) on another pituitary. Flattening was carried out on mercury in this experiment. The valuation of the staining intensity in the control pituitary half is + + for the transitional lobe and ± for the pars intermedia.

TABLE IV

Fixation in formolgas in hours	24	48	72
PAS staining intensity in:			
transitional lobe	+ +	+ +	+ +
pars intermedia	±	±	±

Besides the PAS reaction other staining reactions were carried out on adaequately fixed (24 h.) sections. Fitting in the whole of this investigation the Azan staining (modification according to Heidenhain) and the AF staining according to Gomori may be mentioned. Good staining results could be obtained.

[1] Acetonitril (methylcyanide) does not give rise to dislocation or dissolution, this has been tested after adequate formolgas fixation. Under the present circumstances (amount of paraformaldehyde and size of the vessel) the critical fixation time appeared to be 24 hours for the glycoproteins in the transitional lobe.

Knowing that formolgas fixation on deparaffinized tissue sections gives a good preservation of the glycoproteins and other basophilic and acidophilic substances in the Stickleback's pituitary, it was argued, that even the action of hot xylene and its evaporation could be omitted before fixation, leaving, at least theoretically, the sections in a still more undenaturated state. The paraffin section can be regarded as being porous, leaving sufficient reactive sites of the protein molecules open for reaction with formaldehyde. In this way the solubility of the cell substances could be sufficiently reduced to stay in the section when it is deparaffinized and graded down to water after fixation.

To investigate this possibility, the experiment recorded in table v is set up. Flattening is carried out on mercury. The sections are affixed to "dried" albuminized slides. Preservation of the glycoproteins is revealed by the PAS reaction. The maximum staining intensity reached is indicated by + + for the transitional lobe by ± for the pars intermedia.

TABLE V

Fixation in formolgas in hours	24	48	72
PAS staining intensity in:			
transitional lobe	[illegible]	+ +	+ −
pars intermedia	±	±	±

Here the critical fixation time appeared to be 48 hours.

3. *Discussion*

As can be seen in table III 24 hours can be regarded as the critical fixation time, if fixation is applied to deparaffinized sections. The glycoproteins are then brought in a sufficiently insoluble state to prevent dislocation in or dissolution out of the section.

Cytologically no influence of a too long fixation time is observed comparing sections from tissue pieces, which were fixed in toto, with formolgas fixed sections, neither after the Azan staining, the PAS reaction, nor the AF staining.

One peculiarity observed in the time series experiment deserves attention: at the shorter fixation times dislocation of the PAS positive materials is seen in the pars intermedia ($\leqslant$ 4 hours), and in the transitional lobe (after 16 hours) indicating, as is also revealed by the intensity of the staining reaction in the transitional lobe, that the solubility of the cellsubstances is not sufficiently decreased at these times. In the pars intermedia the glycoproteins no longer are distributed homogeneously over the cells, but have migrated to the cell membrane,

showing a certain affinity for it. This picture is clearest in the cells of the pars intermedia if no fixation at all is applied.

The possibility of showing glycoprotein in the pars intermedia, however dislocated, and the impossibility of showing these substances in the transitional lobe in unfixed sections, indicates a different solubility of the respective glycoproteins (at least after freeze drying).

For gas fixation on undeparaffinized sections, the critical fixation time is about twice as long as for deparaffinized sections. This can be explained as a result of bad diffusibility of the gaseous formaldehyde if the section is impregnated with paraffin.

IV. CYTOLOGICAL DESCRIPTION OF THE HYPOPHYSIS OF GASTEROSTEUS ACULEATUS FORMA TRACHURA BASED ON THE RESULTS OF VARIOUS STAINING METHODS

A. INTRODUCTION

Antecedent to a description of the different cell types which can be observed in the pituitary of *Gasterosteus aculeatus* L. forma *trachura* Cuv., some general remarks should be made.

The pituitary, which measures maximally $1.2 \times 0.7 \times 0.7$ mm is situated ventrally and far rostrally against the tuber cinereum of the diencephalon (see fig. 2). It lies between both lobi inferiores. Somewhat more caudally the saccus vasculosus is met with. Further frontally the chiasma nervorum opticorum is found. In a longitudinal section a cavity, which is situated dorsally of the pituitary, is seen. This is apart of the cavity in the diencephalon, the cavity of the infundibulum, which has the shape of an inverted T in frontal section (see fig. 3). Both laterally directed evaginations of the infundibular cavity are called recessus infundibuli. Moreover, the infundibular cavity has an evagination, which penetrates the pituitary: the recessus hypophyseos (see fig. 4a). In longitudinal section (fig. 4), the pituitary seems to be suspended from the brain by 2 connecting strands of tissue:

1. a thin rostral connection which consists of fibrous material and which is covered rostrally with connective tissue and caudally with ependyma, and

2. a thick caudal one, which is covered caudally with connective tissue and rostrally with ependyma. Besides by its volume this connecting tissue can strike by its abundance of colloid filled spaces.

Three dimensionally these connections form a very short hollow tube connecting the hypophysis with the brain (pituitary stalk). Dorsally, between both connections, the pituitary is covered with ependyma

At the outer surface the pituitary is surrounded by a connective tissue capsule which is continuous with the periost and the dura mater.

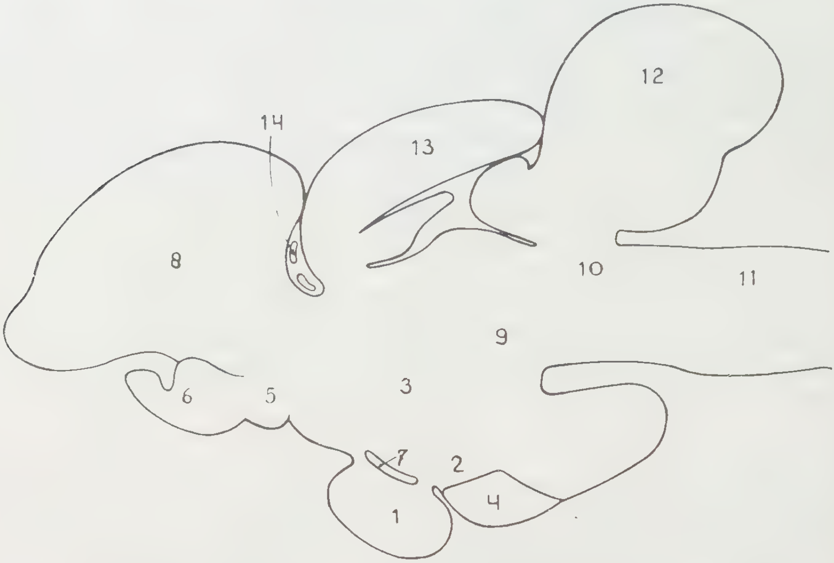


Fig. 2. Schematic longitudinal section through the brain and the hypophysis. 1. Pituitary; 2. Tuber cinereum; 3. Diencephalon; 4. Saccus vasculosus; 5. Chiasma nervorum opticorum; 6. Nervus opticus; 7. Recessus infundibuli (the infundibular cavity is not shown in its vertical expanse as it is very narrow); 8. Telencephalon; 9. Mesencephalon; 10. Metencephalon; 11. Myelencephalon; 12. Cerebellum; 13. Tectum opticum; 14. Epiphysis.

Turning now to the microscopical anatomy of the pituitary we can distinguish between 4 different parts (fig. 4a). The names used here for these parts are those given by STENDELL (1914) to similar parts in pituitaries of other fishes, whereas BOCK (1928) also used this terminology.

In a longitudinal section the following parts, the first three of which are regarded as originating from the roof of the mouth (adenohypophysis), can easily be seen at low magnification:

a. The pars anterior is the more rostral part of the pituitary. A lobe of pars anterior tissue runs ventrally under the transitional lobe. Spatially the pars anterior does not only lie against the transitional lobe, but surrounds the transitional lobe rostro-ventrally and ventrally to some extent.

b. The transitional lobe (the "Übergangsteil" in the German literature) is found in the centre of the pituitary. Nowhere it is in connection with the connective tissue capsule.

c. Caudally and at the same time ventrally the pars intermedia is found, which has a rostrally directed extension running ventrally under the transitional lobe.

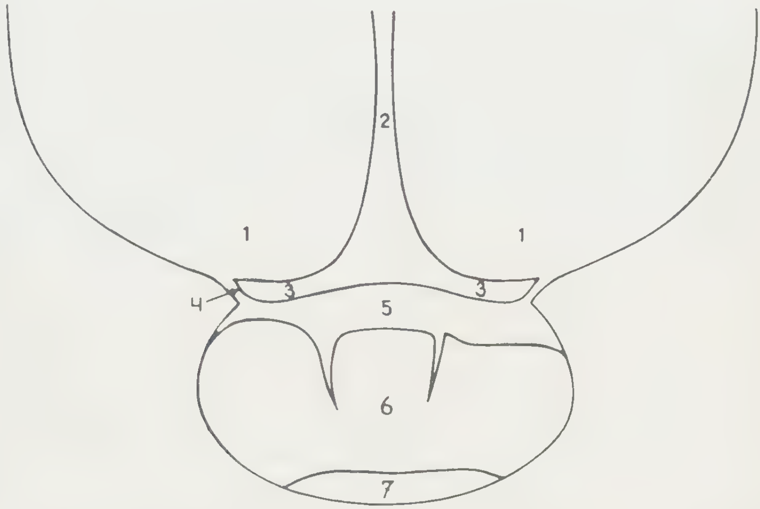


Fig. 3. Schematic frontal section through part of the diencephalon and the pituitary, somewhat rostral of the middle of the pituitary. 1. Tuber cinereum; 2. Infundibular cavity; 3. Recessus infundibuli; 4. Pituitary stalk; 5. Pars nervosa; 6. Transitional lobe; 7. Pars anterior.

d. Dorsally, covering all other parts, the pars nervosa is found. Moreover, offshoots of this part penetrate the other parts. Short, narrow, blunt shoots give a tortuous shape to the dorsal border of the pars anterior. Very fine, long strands penetrate the transitional lobe, whereas a long, more or less broad strand penetrates between the pars anterior and the transitional lobe. Very bulky shoots penetrate the pars intermedia, giving a very tortuous shape to it. In their turn these shoots give off fine strands of tissue which run between the cells of the pars intermedia.

The foregoing is a representative picture of the division in "partes". Besides by differences due to a more or less deep penetration of the broad offshoots of the pars nervosa and those due to the variability in the size of the surfaces of the different lobes, the picture can be different by the position of the border between the pars anterior and the pars intermedia. This border can be situated more caudally (see fig. 4b, this is the situation given by Bock, 1928), but sometimes even a transitional lobe is found which also separates both these parts in the ventral part of the pituitary (see fig. 4c).

Preceding the description of the cell types a few remarks can now be made:

1. sex differences are not obserred,

2. differences between animals which are caught in brackish water ("Hollands Diep" and "Noordhollands Kanaal") and fishes caught in fresh water ("Oude Maas") are not found,

3. all nuclei stain faintly with all stains used. Only iron hematoxylin (Heidenhain) is able to give a somewhat stronger nuclear staining,

4. with one doubtful exception it can be said, that mitoses have never been found in pituitary cells. Only once a nucleus in mitosis was observed in the transitional lobe, but it was situated so close to the connective tissue capsule, that it could have been a nucleus of a fibroblast.

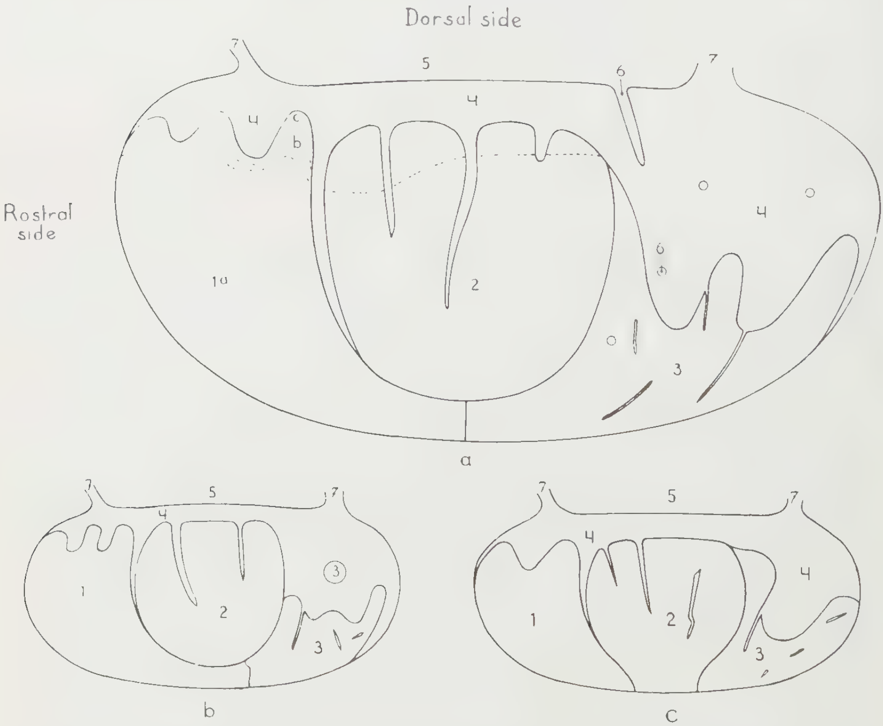


Fig. 4. Schematic medial section through three different pituitaries, a, b and c showing variability in the boundaries of the different parts. The borders of the parts are marked by full lines, the areas within the parts by broken lines. 1. Pars anterior (a. acidophilic cell area, b. chromophobic cell area (if present), c. area of the dorsal lining cells); 2. Transitional lobe; dorsally of the broken line the dorsal zone, if present, can be found; 3. Pars intermedia; 4. Pars nervosa; 5. Recessus infundibuli; 6. Recessus hypophyseos; 7. Pituitary stalk.

B. PARS ANTERIOR

The rostral part of the pituitary (pars anterior) consists for the major part of acidophilic cells. These give it a rather homogeneous outlook. However, on closer inspection more cell types than acidophiles only can be distinguished. Then the parts anterior appears to consist of the following three cell areas (see fig. 4a) with the following cell types:

the acidophilic cell area covering the entire pars anterior, except dorsally, which is composed of:

1. acidophilic cells
2. chromophobic cells,

the dorsal lining cell area, which lies farthest dorsally and which consists only of

3. dorsal lining cells,

the chromophobic cell area, which is situated between the acidophilic cell area and the dorsal lining cells and which is only composed of:

4. chromophobic cells.

These cells whill be discussed in the order given. After this the blood supply of the pars anterior will be treated and some remarks on the so called basophilic bodies will be made.

1. *Acidophilic cells* (see Plate III–1)

These cells stain red with acid fuchsin or azocarmine in the Mallory or Azan staining respectively. The HE staining also gives expression to the acidophila by strongly binding eosin, which is, however, less distinct. Still somewhat less distinctly the acidophilia is demonstrated by the panoptic staining according to Pappenheim.

As a rule the shape of the acidophilic cells is more or less oval. Round or nearly round cells however, are to be found as well, while also some cells of a more angular shape can be present. Their shape is nearly round or oval in the case the cells are detached. Many intercellular spaces, which can have great dimensions, can be met with. Only the acidophiles which are found against the connective tissue capsule and against the basement membrane in the caudal portion of the pars anterior, have a more elongated shape. They are situated against the capsule or the basement membrane with their shorter sides. Cell borders can be seen clearly. The size of the cells is about 4×5 to about $9 \times 13\ \mu$.

The nuclei of the acidophiles are large and round to oval in shape whereas some bean shaped nuclei can be found, sometimes even some with invaginations or indentations. The nuclear dimensions vary from about $2.5 \times 3.5\ \mu$ to about $3.5 \times 6.0\ \mu$. They possess dictinct chromatin

networks, and they are only slightly tinged. Nucleoli can be seen after taining with iron hematoxylin (Heidenhain). The protoplasm of the acidophilic cells appears to contain the particulars to be described now.

a. *Acidophilic material.* The cytoplasm appears to stain (homogeneously) red in most cases in 5 μ thick sections after Azan, Mallory, HE or CHP staining. Hence it contains acidophilic material. Occasionally cells can be found in which part of the cytoplasm is stained darker than elsewhere in the same cell. After staining with the above mentioned methods only few granules are found. An occasional thin offshoot can show distinct granules. Granules were also seen in 1 μ thick sections, but here too far from all material was seen to be localized in granules.

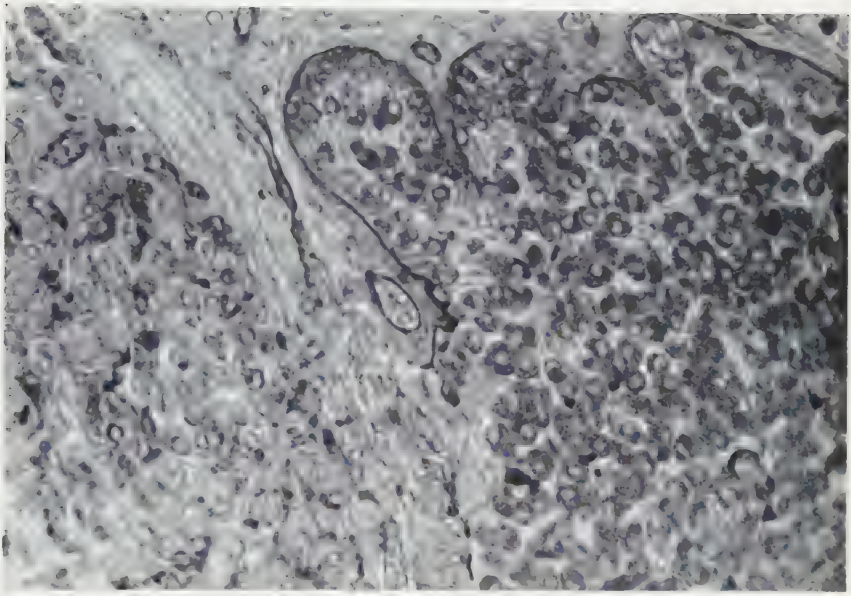
In the acidophiles of one animal only very large granules (up to 1.5 μ) were found after CHP staining. Some cells were densely filled, some showed only a few granules per cell, whereas others, having the morphological appearance of acidophilic cells, were completely devoid of acidophilic material. This was the only case in which acidophilic cells, only partly filled with acidophilic material, were observed.

The acidophilic material is completely PAS and AF negative.

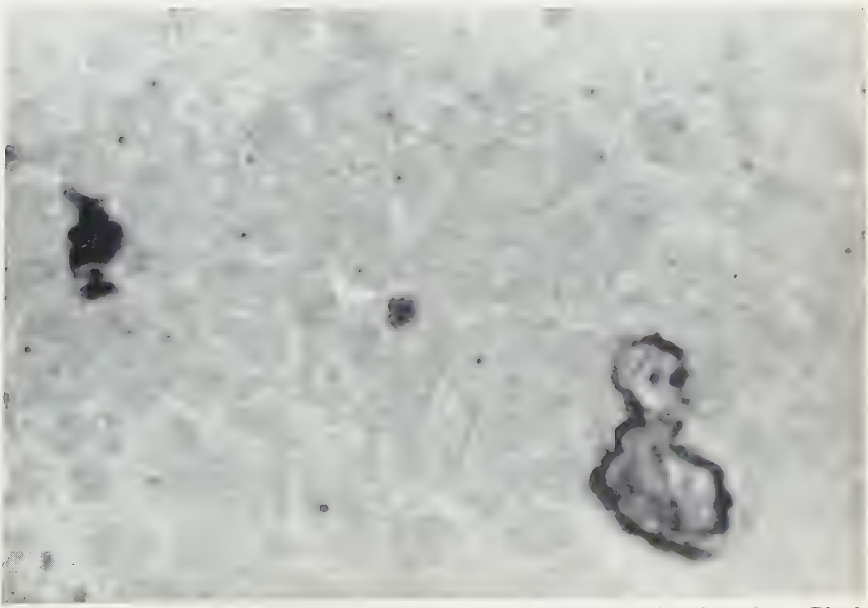
b. *Juxtanuclear granules.* This granulation is blue after Azan staining. After the PAS reaction they appear to be positive (see Plate III–2 and IV–1) and stain transparent red. They seem to contain glycoprotein (see p. 175) The juxtanuclear granules (centrioles) vary in diameter from about 1 to 1.4 μ, consequently they are considerably larger than the acidophilic granules. Sometimes the juxtanuclear granule lies against the nucleus, but usually somewhat removed from it. Not all specimens seem to possess this granulation. They are clearly demonstrable after formol sublimate fixation and after freeze drying followed by formolgas fixation. They can be found with difficulty only after Zenker acetic fixation, whereas after freeze drying without fixation they are not to be found. In the material of the two halves experiment, described in chapter III, it is clearly observed that the pars anterior of the half which is fixed with formolgas after freeze drying contains juxtanuclear granules, whereas the other half of the same pituitary, which is not fixed, does not show these granules.

Generally it can be said that these granules are inconspicuous. If this granulation has been seen after the PAS staining reaction, it can be found again with some difficulty after Azan staining. It is probably due to this peculiarity and to the fact that at the time of Bock's (1928) paper on the Stickleback's pituitary the PAS reaction was not known, that Bock has not seen this granulation; anyhow, Bock has not described it. The juxtanuclear granule appears to be AF and Feulgen negative. Also after panoptic staining, CHP, HE and iron hematoxylin (Heidenhain) it is not to be observed.

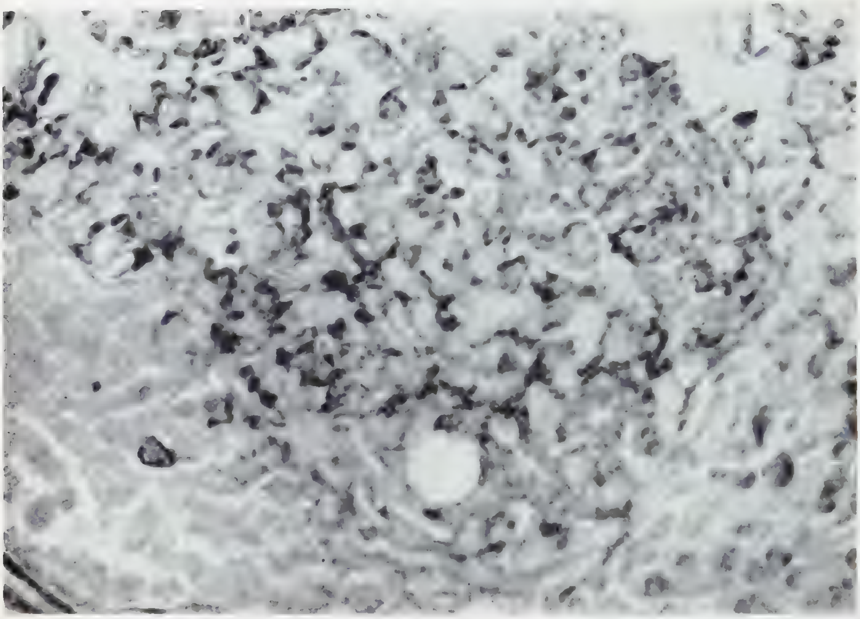
PLATE III



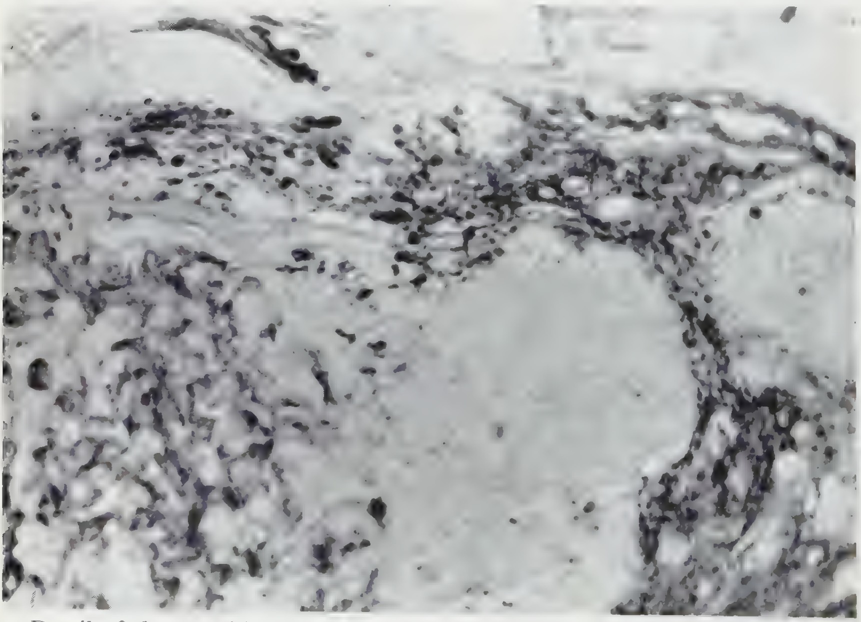
1. Detail of the pars anterior, the transitional lobe and the pars nervosa. The red acidophiles of the pars anterior are rendered dark grey. Some basophilic bodies between these cells (black dots). The purple dorsal lining cells do not contrast greatly with the acidophiles. Some chromophobes are visible. A basement membrane borders the dorsal lining cells. The transitional lobe shows the yellow acidophilic cells as light grey. Dark grey: basophilic cells. Some sections through blood vessels are found, mainly in the pars nervosa. Fixation: Zenker acetic. Azan. × 545.



2. Detail of the pars anterior. The juxtanuclear granules: black points. Black dots: basophilic bodies. Lower right: section through blood vessel. Fixation: formol sublimate. PAS. × 425.



1. Detail of the pars anterior (lower left), the transitional lobe and the pars intermedia (lower right). Black and dark grey: PAS positivity (glycoproteins). In some cells of the pars anterior juxtanuclear granules can be seen. Fixation: formol sublimate. PAS. × 430.



2. Detail of the transitional lobe (lower left), the pars intermedia and the pars nervosa (right). The pars nervosa is filled with neurosecretory substance (black). Threads of neurosecretory substance are seen in the caudal connection of the pituitary to the diencephalon. Some sections through the recessus hypophyseos are visible in the pars nervosa. Freeze drying followed by formol gasfixation. AF. 465.

c. *RNA*. After Panoptic staining in most cells the nucleus is surrounded by a narrow blue band, which in a few instances only shows distinct granules. Locally greater concentrations of this material in which generally no granules can be seen light microscopically are deposited against the nucleus. Often, but not always, these patches lie at that side of the nucleus where the smallest quantity of cytoplasm is to be found. Sometimes a narrow zone is seen along the cellborder. If granules are present, which are visible both after formol sublimate and Zenker acetic and after freezedrying, whether followed by formolgas fixation or not (in the last instance less clearly), they have about the same dimensions as the acidophilic granules, so up to about 0.8 μ.

The material described here can be easily observed after panoptic staining, adequate Azan staining, iron hematoxylin (Heidenhain) and methylene blue staining at pH's of 4 and higher, but only vaguely after HE staining. Moreover this material can be made visible by fluorescence microscopy after staining with acridin-orange at pH's of 4 and higher as brown red masses. According to recent evidence (VON BERTALANFFY and BICKIS, 1956) cell inclusions which fluoresce red or brown red with acridin orange can be considered to consist mainly of ribonucleic acid (RNA). This has appeared from the staining behaviour of these inclusions after ribonuclease (RNase) treatment and from the staining results of commercial RNA in test tube experiments. Moreover such material stains with basic dyes as toluidine blue and azure B (BRACHET, 1957). Of this group of dyes (the thiazins) methylene blue is used in this investigation to stain RNA. This dye can be considered as tetramethylthionin, whereas azure B and toluidine blue are trimethyl- and dimethylthionin respectively. With methylene blue pictures similar to those after acridin orange staining are obtained. As substitute for RNase, since RNase itself was not at the author's disposal, HCl has been used. According to FISHER (1953) HCl, HNO_3, H_2SO_4 and perchloric acid destroy selectively cytoplasmic basophilia originating from RNA under certain circumstances. At 4°C a 6 hours treatment with HCl at 2 M concentration removes all RNA from the pituitary cells, as is revealed by methylene blue staining. The above mentioned facts make it likely that the material discussed here contains RNA.

2. *Chromophobic cells*

While by far the greater part of the acidophilic cell area consists of acidophilic cells, some chromophobic cells can be present scattered between them (see Plate III–1). These cells have fully unstained cytoplasm after the Azan staining method. They seem to have no charac-

teristic shape; they occupy spaces left open between the acidophiles. Cell borders are inconspicuous. The nuclei are round to oval, but often flattened nuclei are found; they are of the same dimensions as the nuclei in the acidophiles. Juxtanuclear granules are not found in these cells. RNA is only met with in small amounts around the nuclei after the staining methods described for the acidophiles.

3. *Dorsal lining cells*

The pars anterior is not exclusively filled with the above described acidophilic and chromophobic cells. At the dorsal side of the anterior part a number of more or less distinctly epithelially arranged cells of another type can be observed (see Plate III–1). After Azan staining these cylindrical cells have a purple colour and owing to this they stand out from the acidophiles, a distinction which also becomes evident in the HE and CHP stainings by the greyish rose and greyish red colours respectively.

The nuclei of the dorsal lining cells are generally large in comparison to those of the acidophilic cells. As a rule they are round to oblong oval; bean shaped or invaginated nuclei are absent. They possess a distinct chromatin network. Nucleoli were found only after iron hematoxylin (Heidenhain) staining.

A distinct basement membrane, which is stained blue after Azan, red after PAS and faintly purple after AF, constitutes the border between these cells and the pars nervosa (see Plate III–1). The basement membrane rostrally merges into the connective tissue capsule which surrounds the hypophysis rostrally, ventrally and caudally. From rostrally the membrane runs caudally, following exactly the tortuosities originating from evaginations of the pars nervosa into the pars anterior. On the spot where a broad strand of the nervosa tissue penetrates between the pars anterior and the transitional lobe, this membrane bends ventrally. From here it can follow its way over a distance of about half the height of the pituitary. This part of the basement membrane is no longer in contact with the dorsal lining cells, but with the acidophiles of the pars anterior. Still further ventrally the strand of nervosa tissue is in direct contact with the cells of the pars anterior: the basement membrane is lacking here. Intimate contact between nervosa and pars anterior tissue is established wherever the basement membrane is interrupted over short distances. Local interruptions of the basement membrane are the rule rather than the exception. In some sections only a continuous basement membrane can be observed.

Occasionally an entirely acidophilic cell is found between the dorsal lining cells.

a. *Amphoteric material.* The contents of the epithelially arranged cells are made up of granules and small clumps, which stain purple in the Azan staining. The cells are less densely filled than the acidophiles. This material takes up the azocarmine as well as the anilin blue. So in staining properties it stands in between acidophilic material and basophilic material and is called amphoteric.

b. *Juxtanuclear granules.* In the dorsal lining cell one can find no or only in a few instances a basophilic PAS positive juxtanuclear granule, as opposed to what is the case in the acidophilic cell.

c. *RNA.* In this celltype an amount of material is found which is stained as RNA in the panoptic and methylene blue staining at pH's of 4 and higher. After staining with acridin orange at pH's of 4 and higher it demonstrates a reddish-brown fluorescence. The stained spots, which can be partly granular are much larger here than in the acidophilic cells. A distinction in the amount of RNA in this sense was very clearly met with in a pituitary in which no RNA appeared to be present in the acidophiles, whereas in the dorsal lining cells large accumulations around the nuclei were found.

4. *Chromophobic cells of the chromophobic cell area*

Usually, although not always, the area of the acidophiles is separated from the epithelially arranged cells by a layer of chromophobic cells (see Plate III 1). The fact that the protoplasm of these cells is nearly colourless after e.g. the Azan staining, makes the mass of protoplasm around the nuclei difficult to distinguish. This strand of tissue consists of 1–3 layers of cells. Cell borders are difficult to see. Transitional forms between purple dorsal lining cells and chromophobes, and between chromophobes and red acidophiles can be observed. The nuclei in this zone are large in comparison to those of the acidophilic cells. They are also round to oblongly oval, without indentations. There is a distinct chromatin network. Nucleoli are only observed after iron hematoxylin (Heidenhain) staining.

a. *Juxtanuclear granules.* In these chromophobes only sporadically a granule is met with, which is not as clearly PAS positive as that in the acidophilic cells.

b. *RNA.* The bands and accumulations of RNA which are described for the acidophiles are rarely found in the chromophobic cells after panoptic, methylene blue and acridin orange staining. In a few instances there is a band around the nucleus.

The blood supply of the pars anterior originates from the connective tissue capsule, from the pars nervosa, dorsally of the dorsal lining cells,

and from the strand of nervosa tissue situated between the pars anterior and the transitional lobe (see Plate III–1). In most sections several bloodvessels are to be found; if more of them are present, they are rather regularly scattered over the pars anterior. Nuclei of the lining endothelium are found only rarely. The wall consists of connective tissue which stains intensely blue in the Azan and Mallory technique and which is PAS and very faintly AF positive. Nowhere, however, a distinct arrangement of acidophiles around the bloodvessels is observed, as STENDELL (1914) describes in *Esox*. In the last named case the cells adhere to the bloodvessels with their narrow sides. In *Gasterosteus aculeatus* forma *trachura* no aberrant cell shapes are found in the neighbourhood of bloodvessels.

BOCK (1928) in his paper on the forma *leiura* has described a cell arrangement around the bloodvessels as is mentioned above for *Esox*. To study the discrepancy between Bock's findings and ours some pituitaries of *Gasterosteus aculeatus* forma *leiura* were sectioned and investigated on the point of blood supply of the pars anterior. Indeed, oblong acidophilic cells around the bloodvessels are found here, the long axis of the cells arranged radially.

The curious structures named basophilic bodies, found in the acidophilic cell area, deserve mention now (see Plate III–1). Often one can find patches which are blue after Azan and red after PAS. From these patches often go out very short, fine strands of anilin blue stained material. To a few of such basophilic bodies one or two small colourless cells are attached. These cells contain small nuclei. This picture morphologically ressembles that of the Hassall-bodies in the thymus.

C. TRANSITIONAL LOBE

Judging from the nature of the histological picture the border of the transitional lobe is easily drawn at low magnification. However, the borderline between this part and the pars intermedia, especially at higher magnification, is not always a very sharp one.

In the transitional lobe three different celltypes occur: acidophiles, basophiles and chromophobes.

1. *Acidophilic cells*

These cells strike immediately by their size, shape and colour (see Plate III–1). They are for the greater part oval, although some round or more or less angular cells can be found. The dimensions of these cells vary from about $5 \times 6\ \mu$ to about $8.5 \times 11\ \mu$.

The nuclei of this celltype vary in shape from round to oval, but

bean shaped nuclei with more or less deep, narrow and broad invaginations are to be met with as well. Moreover a number of nuclei with indentations is found (see fig. 5 and Plate III–1). A few nuclei can be a little more angular in shape. Nuclei with invaginations or indentations belong to the larger ones. In a few instances a binucleated cell is observed. All possess a distinct chromatin network. The diameter of the round nuclei lies between 2.5 and 5.0 μ.

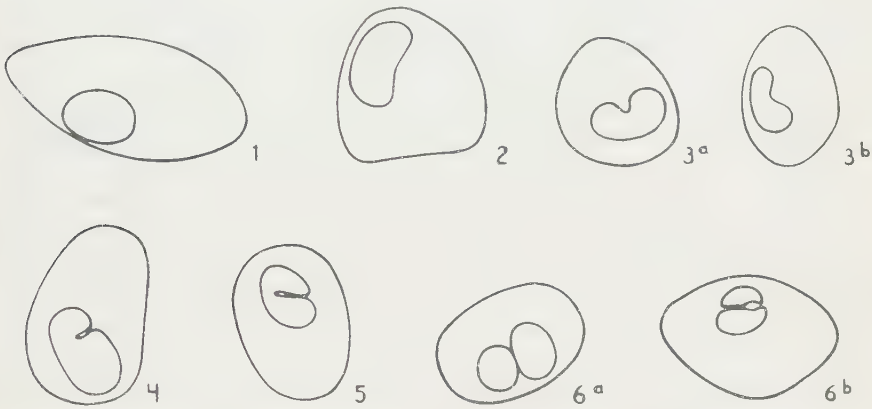


Fig. 5. Variability in the shapes of nuclei in acidophilic cells of the transitional lobe. 1–6a are found in one section, 6b in another section of the same specimen. 1. rounded nucleus; 2–3b. nuclei with invaginations; 4–5. nuclei with indentations; 6. binucleated cells.

Observation of these cells at higher magnification reveals some details. Here too three types of protoplasmatic material will be discussed:

a. *Acidophilic material.* This material accounts for the general staining capacity of the cell. In most sections it seems to be distributed diffusely over the cells. However in a few cases granules are visible, the size of the largest one being near limit of resolution of the microscope.

The colour of the acidophilic cells varies from red through orange yellow to yellow after adequate Azan staining. They can consequently be considered acidophilic in character. However, sometimes a definite difference in staining capacity can be remarked between the acidophiles of the anterior part and those of the transitional lobe. The acidophiles of the pars anterior always (with one exception) take up the azocarmine and are stained intensely red, whereas the acidophiles of the transitional lobe can be stained either red or yellow or can show an intermediate colour. This varies with the various individuals and is found fairly often. Within one and the same individual always

the same colour is present in the areas separately. In one case only it was observed that, although the transitional lobe stained yellow, one of its acidophiles stained red, just as the cells of the pars anterior.

The one exception mentioned on p. 173 concerns a pituitary in which the acidophiles of the pars anterior were stained yellow, those of the transitional lobe stained red: just the reverse as usually found.

b. *Juxtanuclear granules.* Just as in the acidophilic cells of the pars anterior, one PAS positive granule per cell can be found in the transitional lobe. Here too this granulation is not met with in every specimen. It is situated against the nucleus or at a small distance from it.

If these granules are stained with PAS, they are difficult to find, because of possible confusion with other granules of PAS positive material, which can be present in the transitional lobe (in the basophiles, see below): after PAS staining cell shapes can be determined with difficulty only. However, if the acidophiles stain yellow in the Azan staining, these granules are easily to be seen due to the good contrast: yellow–dark blue. The presence of the juxtanuclear granule after different fixations and its stainability after Feulgen, panoptic and AF staining is the same as mentioned for the acidophiles of the pars anterior.

c. *RNA.* RNA is met with in these cells to about the same amount as in the acidophiles of the pars anterior. The description given for this material in these cells is valid here as well.

2. *Basophilic cells*

After the Azan technique the basophiles stain blue (see Plate III–1). Often certain parts of the cell, sometimes in the neighbourhood of the nucleus, appear to be darker toned.

Granulation is not obvious after this staining method. Cell borders withdraw from observation after most staining procedures. Sometimes, after a regressive iron hematoxylin (Heidenhain) staining, they are visible. In a few instances a cell is so crowded with strongly stained basophilic material that this phenomenon betrays the shape of the cell. Sometimes a cell is found with slightly vacuolated protoplasm. In the HE and CHP stained sections basophilic cells can be distinguished from the acidophiles on the account of their greyish colour. More details of these cells will be discussed along with those of the chromophobic cells (see below).

3. *Chromophobic cells*

Again a Mallory type staining, e.g. the Azan staining, reveals this cell type (see Plate III–1). The chromophobe is a colourless or faintly

blue cell. Some very thin threads and fine granules are visible in its cytoplasm. Cell borders are mostly invisible.

Although after HE or CHP staining the chromophobes can be distinguished from the acidophiles in this area, just as the basophiles can be, chromophobes and basophiles mutually are not to be distinguished from one another after these staining methods. As we have seen above, however, after the Azan staining distinction can be made between typical basophilic and chromophobic cells. Many transitions between them can be observed. For this reason the following particulars will be given without making a sharp distinction between basophiles and chromophobes.

Nuclei are round to oval, some are bean shaped. Some nuclei with invaginations and indentations can be found as well. They possess a distinct chromatin network, nucleoli are seen only after iron hematoxylin (Heidenhain). Some nuclei are stained somewhat lighter, some somewhat darker than the others. The nuclear diameter is about 3.5 μ.

Juxtanuclear granules are not observed in these cells since no distinction can be made between these granules, if present, and the secretory material as both are red after PAS and blue after Azan staining. In the sections in which the PAS positive material was washed out (blancs in the formolgas time series, see chapter III) this granulation is not found either. This is not entirely surprising since the juxtanuclear granules behave themselves similar to the PAS positive secretory material in the tests mentioned below. Thus the juxtanuclear granules seem to contain (readily soluble) glycoprotein.

RNA could be demonstrated clearly by acridin orange at pH's of 4 and higher, methylene blue at pH's of 4 or higher and after the panoptic staining according to Pappenheim. However, the accumulations are not as large as in the acidophilic cells and not all cells contain RNA.

The PAS reaction reveals an interesting phenomenon in the chromophobic and basophilic cells. Many granules and clumps of PAS positive material, which stain darkly, can be observed (see plates I–1, I–2 and IV–1). These patches can have irregular shapes. Granules and patches can be stained in different intensities, even within one and the same clump. A single cell can be completely filled with positive material. Between liquid fixed sections and frozen dried sections a difference is observed: more and distinct granules are observed after freeze drying and formolgas fixation (chapter III).

This PAS positive material is considered to be glycoprotein. According to the advises given by DANIELLI (1953) and PEARSE (1953) the following tests exclude substances which can also give a positive Schiff reaction:

1. Free aldehydes are not present in the sections used for the PAS technique, because after exposure to Schiff's reagent without previous periodic oxidation no staining is brought about.

2. Acetal aldehydes can be excluded as after 0.1 N HCl during 15 min. in the cold followed by Schiff's reagent no Schiff positivity can be observed.

3. Of the Schiff positive tissue components which can be demonstrated after oxidation, glycolipids, unsaturated lipids and phospholipids can be excluded as lipid extraction with hot ether does not influence the strength of PAS positivity.

4. Glycogen is not present in the sections as they are fast to half an hour treatment with ptyalin at 24° and 37° C. No distinction is made between the other substances which can give a positive PAS reaction (neutral mucopolysaccharides, mucoproteins and glycoproteins).

That aldehyde groups are involved in the Schiff reaction after periodic acid oxidation is tested by a blocking reaction. Dimedone, a specific reagent for aldehydes (PEARSE, 1953), abolishes a positive Schiff reaction in 6 hours, in a saturated alcoholic solution.

The question could be raised whether or not be PAS positive material has the same localization as the strongly blue staining material. To investigate this, sections from 5 animals, which had been kept under the same circumstances in the laboratory, were first stained with Mallory. Coloured drawings were made on transparent paper by means of a microscope with oil immersion objective and a camera lucida. After removal of the dyes (alcohol) the sections were restained by the PAS procedure and again a coloured drawing was made. Some other sections were used in a similar comparison experiment with photomicrography. These experiments decisively show that, generally speaking, heavy staining after PAS corresponds to dark blue shades after Mallory and slight PAS positivity corresponds with faint blue. However, to a much smaller extent, the correlations of heavy PAS red—slight Mallory blue and slight PAS red—heavy Mallory blue are realized as well.

Another peculiarity, which is observed after PAS staining, deserves mention now. In sections from some pituitaries it can be observed that the localization of the positive material in the dorsal part (see fig. 4a) of the transitional lobe was slightly different from that in the rest of this lobe: while the clumps in the large ventral part can be very large and of irregular shape, the clumps in the dorsal part are smaller and have a more elongated shape. Sometimes they are even rodlike. The clumps and granules lie close together and their colour density is lower than in the rest of the transitional lobe. An aberrant dorsal part could also be seen after Azan staining.

Treatment of a PAS stained section with methyl blue leaves the colour of the PAS positive material in the transitional lobe unchanged.

At a first glance the AF staining method seems to reveal the same material as the PAS method (see plate IV–2), irrespective of the oxidizing agent used for the AF staining ($\frac{1}{2}$% I_2 in alc. 70% during up to 6 hours, Lugol during up to 4 hours according to Halmi's method, $HgCl_2$ during up to 24 hours or $KMnO_4$–H_2SO_4 during 1 min.).

To test this hypothesis the following experiment is set up: two photomicrographs were made with an oil immersion objective with the highest available numerical aperture (1.40), each composed of 10 single photomicrographs to cover the whole area of the transitional lobe. The first photomicrograph was made after PAS staining, the second after AF staining. This sequence was chosen because the PAS staining is removed by oxidation with $KMnO_4$ H_2SO_4 during 1 min., this being part of the AF procedure. Control experiments were performed to study the stainability with AF solution of the sections previously oxidised with periodic acid during 10 min., as is done in the PAS routine. No or a very faint staining is brought about by the treatment with AF solution during 1 ¼ min., as it is done during the second staining in the photomicrographic experiment, after 10 min. periodic acid oxidation.

Moreover oxidation by treatment with periodic acid during 10 min. followed by $KMnO_4$–H_2SO_4 during 1 min. reveals the same pattern of AF positivity as single oxidation by $KMnO_4$–H_2SO_4 during 1 min.

In the resulting photomicrographs (× 765), size about 50 × 60 cm., 29 circular spots, evenly distributed, which have a diameter of about 38 mm., were chosen and the percentage of correlation between AF and PAS positive granules and clumps estimated by counting. This percentage appeared to be 82.4 .The remaining 17.6 % of AF positivity not found back as PAS positivity in the other photomicrograph is regarded to be due, for the greater part, to an unequality in focal plane when both photographs are made and to slight distortions in the tissue after the second staining. Moreover, while counting a few PAS positive clumps were met with, which were not found back as AF positive clumps.

To avoid focal mistakes in 7 other pituitaries only the section after PAS staining was photographed, this photograph being compared with the section after restaining with AF. This reveals that only a very few granules or clumps can be found, which are AF positive, but not PAS positive. Here too little PAS positivity was not found back as AF positivity. From these experiments it can not be concluded that the PAS positive material is differently localized from the AF positive material: with a few exceptions the localization of the granules and

clumps is the same after both staining procedures. However, it is not settled, whether two different substances occur mixed and are thus being produced within the same cell type or that one cell substance is stained by both staining methods.

In the first experiment it could be established that a certain dorsal zone of the transitional lobe (fig. 4a) was composed of significantly (χ^2 test on counts, 5 % limit) more granules and clumps per surface area than in the rest of the transitional lobe. On this ground, beside the already mentioned morphological differences of the PAS positive material, the dorsal zone can be distinguished from the rest of the transitional lobe. However, the borders are not sharply defined and not in all animals a distinct zone can be observed.

D. PARS INTERMEDIA

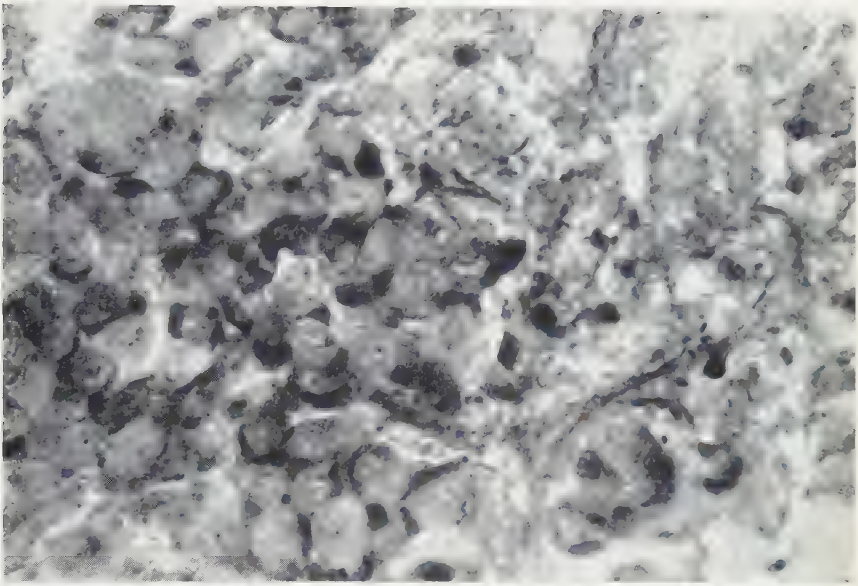
This cell area, which just as the above described parts originates ontogenetically from the roof of the mouth, distinguishes itself clearly from the other parts on account of cell shape, cell size, stainability and the whole nature this area. In connection with its stainability it shows after Azan staining two cell types: the more chromophilic cells (cells with a higher developed chromophilia) and the less chromophilic cells (see plate V–1).

If staining is carried out in such a way as to bring about all the various celltypes in the pars anterior and the transitional lobe well differentiated, then all cells of the pars intermedia, the more as well as the less chromophilic ones, have a dirty red colour. In this case no differentiation is seen. On the other hand, if one stains somewhat longer with the anilin blue-orange G-acetic acid mixture, then a nice range of blue shades is obtained: the less chromophilic cells stand out as bluish red or bluish grey and the more chromophilic cells as blue. As these latter cells have bound more anilin blue, they are called more chromophilic. So the first mentioned method of Azan staining could be called to be adjusted for the two proximal parts of the adenohypophysis, whereas the second is adjusted for the pars intermedia. Not only a range of blue shades between the cells of the two types exists, but within one cell as well: transitional stages between more and less chromophilia.

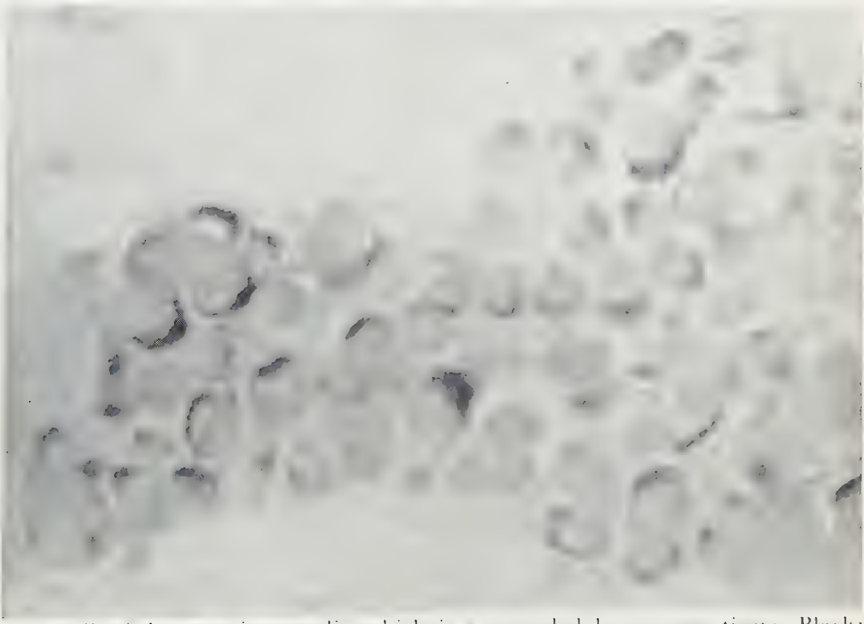
1. *More chromophilic cells*

The cells of this type are present in groups and are found scattered. Their shape is round to oval, but often polygonal shapes are found. The dimensions vary from about 10×7 to about $7 \times 4\ \mu$. The

PLATE V



1. Detail of the pars nervosa (right) and the pars intermedia (left). Black-dark grey: more chromophilic cells, light grey: less chromophilic cells. Top left: some acidophilic cells of the transitional lobe. Fixation: formol sublimate. Azan. × 975.



2. Detail of the pars intermedia which is surrounded by nervosa tissue. Black: RNA. Freeze drying followed by formolgas fixation. Methylene blue. × 1230.

nuclei are round to oval, some showing invaginations or indentations. They possess a distinct chromatin network and vary from about 4.7×4.4 to about $3.1 \times 2.6\ \mu$. Cell borders are usually well visible. After Azan staining no granulation is seen in these cells.

2. *Less chromophilic cells*

In the second place cells are found, which are bluish red or bluish grey in colour. They lie scattered over the pars intermedia, sometimes in groups together. Their shape is round to oval, sometimes polygonal. They measure from about 8×6 to about $6 \times 4.5\ \mu$. Their protoplasm is homogeneous, no granules are found after Azan staining. The nuclei are also round to oval, whereas their average dimensions are about $3.9 \times 2.6\ \mu$. Nuclei which are invaginated and which show sharp indentations can be observed in some pituitaries.

The results obtained with other staining methods with regard to the pars intermedia will now be described. In some instances these results will be brought in relation with the above mentioned cell types.

Juxtanuclear granules could not be detected after Azan staining. This is explicable, as blue granules do not show a great colour contrast with the blue or bluish red or even bluish grey plasm. After the PAS reaction these granules could be detected, but not in all specimens and not in all cells. That juxtanuclear granules can not be found in all cells is at least partly due to lack of colour contrast: some intermedia cells are PAS positive, so a PAS postitive granule can be obscured. From the Azan–PAS staining experiment (see below) it seems legitimate to draw the conclusion that, generally, the juxtanuclear granules, if present, are situated in the less chromophilic cells. Whether the more chromophilic cells too can contain such a granule cannot be said now.

In all cells of the pars intermedia RNA accumulations, and sometimes granules, are to be found around the nucleus. Sometimes they are found against the cell borders as well. This is observed after panoptic staining (according to Pappenheim), methylene blue staining in acid medium (see Plate V–2) and after acridin orange staining. HE shows the localization of this material poorly. In the pituitaries investigated on RNA the greatest accumulations of this material were always found in the intermedia cells.

After all fixation methods used in this investigation a number of cells is met, which, with the exception of the nucleus, stain red after the PAS reaction for glycoproteins. These are usually completely filled with PAS positive material. This material appeared to be less soluble in water (see chapter III). Granules are not to be distinguished. To this 2 animals formed a very well marked exception: they showed discrete

granules in the intermedia after PAS staining. The pituitaries of these specimens were frozen dried, but quenched in Freon 13[1] chilled with liquid air (—180° C). The staining intensity is low in comparison to the intensity of the PAS positive patches in the transitional lobe. Locally a somewhat higher concentration of PAS positive material can be present in the cell, but usually cells are uniform in colour. In some instances it was observed that a narrow zone around the nucleus was free from glycoprotein: a free space in the area in which the RNA can be localized. However in a number of other cases, here and against the cell border a higher colour intensity was found on the contrary.

The question could be raised whether or no a correlation exists between the PAS positive cells and e.g., the more chromophilic cells. To investigate this 302 intermedia cells from 8 sections, originating from 4 animals (kept under the same circumstances in the laboratory) were first judged on chromophilia, later on PAS positivity. For this purpose a coloured drawing of a certain cell area was made, by means of an oil immersion objective and a camera lucida, after Azan staining. After this the sections were destained (alc. 96 %, night over) and restained by the Schiff's reagent after periodic acid oxidation. The same cell area was again drawn in colour. In these drawings the cells were compared[2]. The results of this experiment are given in the following table so as to test the postulate that less chromophilia correlates with PAS negativity and that more chromophilia correlates with PAS positivity.

On account of the high percentages of correlation it can be stated that, generally, a less chromophilic cell appears to be a PAS negative cell and that a more chromophilic cell is a PAS positive one. However, comparison of individual cells in both drawings reveals that the rate of chromophilia does not always correspond directly with the rate of PAS positivity.

After a discussion of the peculiarities of the application of the PAS reaction it is interesting to mention what becomes visible after PAS methyl blue staining. In the Stickleback's pituitary this staining reveals all PAS positive intermedia cells in a purple colour.

Using the AF staining solution the results obtained in the pars intermedia depend upon the oxidizing agent used. If 0.5 % I_2 in alc. 70 % is used for maximally 6 hours, Lugol's solution for maximally 4 hours and a $KMnO_4$–H_2SO_4 mixture during 1 min., the intermedia cells

[1] Freon 13 was used in stead of Freon 12, which is commonly made use of for work at very low temperature, on account of its better heat conducting capacity and its lower freezing point (—180° C), thus preventing the solidification of the Freon during quenching.

[2] The author is greatly indebted to Mr. W. Vink, student of biology at Leiden, who performed the preparative work, made the drawings and carried out the comparisons.

do not stain, whereas in these cases a good positivity in the transitional lobe is obtained. If $HgCl_2$, however, is used in a saturated solution during 24 hours or as a constituent of the fixing solution (formol sublimate) a faint positivity is obtained. In the case mentioned last different staining intensities can be observed between various cells and within one and the same cell.

TABLE VI

Section number	Number of less chromophilic cells	Number of PAS negatives of the less chromophilic cells	% correlation	Number of more chromophilic cells	Number of PAS positives of the more chromophilic cells	% correlation
1	11	11	100.0	26	16	61.5
2	29	20	69.0	16	14	87.5
3	15	15	100.0	19	16	84.2
4	25	20	80.0	17	14	82.3
5	29	24	82.0	19	9	47.4
6	5	5	100.0	23	19	82.6
7	14	13	92.0	26	21	80.8
8	21	18	85.7	7	6	85.7
	Mean % correlation:		88.8	Mean % correlation:		76.5

The pars intermedia as a whole has a tortuous shape because in several places strands of nervosa tissue penetrate dorso-ventrally. In some sections separate groups of cells are met with, isolated from the rest of the pars intermedia by nervosa tissue.

Epithelial arrangement of cells lying against these strands is not found in *Gasterosteus*. According to STENDELL (1914), this is observed in other Teleostei.

Bloodvessels are sporadically met with.

E. PARS NERVOSA

The neurohypophysis, which ontogenetically originates from the floor of the diencephalon and which consists of fibers mainly, is situated dorsally of the adenohypophysis, whereas strands penetrate into the parts of the adenohypophysis in a ventral direction (fig. 4). The more rostral strands dorsally give the pars anterior a tortuous outline (see Plate III-1). A large strand of fibers penetrates somewhat more caudally between the pars anterior and the transistional lobe (see Plate III-1) and some thin strands take their way in dorso-ventral direction between the cells of the transitional lobe. Extremely bulky strands render a tortuous outline to the pars intermedia (see Plate IV-2).

The fibers, which are to be found in this part of the pituitary (see

Plate V-1) with the methods used in this investigation, for the greater part stain just as the fibrous material found in the brain. It is believed that, in the pituitary of the Stickleback, the pars nervosa for the greater part is built up of nerve fibers. It is certain that besides this connective tissue fibers occur, whether in connection with blood vessels or not. These fibers stain intensely blue after Azan.

The course of the fibers is rostro-caudally in the dorsal part, so parallel to the ependyma and the floor of the infundibulum. In the strands which penetrate into the different parts, the fibers mostly run dorso-ventrally. Fibers also occur in the caudal, broad connection of the pituitary with the tuber cinereum. Here the histological picture points to a passage of fibers from the brain to the pars nervosa. In a single instance it was possible to follow fibres over that track, these fibers were filled with neurosecretory substance over their full length.

Comparatively few nuclei are found; they lie scattered over the pars nervosa. Nuclei are met with which have an oblong, more or less drawn out shape, that resemble in appearance the nuclei of fibroblasts and which are normally to deeply stainable with hemalum. Generally, protoplasm could not be detected around these nuclei after HE or Azan. Besides that, some large, round nuclei are found which stain faintly with hemalum. A single smaller nucleus stains deeply and seems to be pycnotic. After HE or Azan staining generally no cytoplasm could be seen in the neighbourhood of these nuclei. Only once a small rest of heavily staining protoplasm was found in a HE stained section.

BOCK's opinion (1928), based on appearance and stainability, is that nuclei which are situated in the pars nervosa, originate from cells of the adenohypophysis, migrating into and dying in the pars nervosa. In this direction point the absence of secretory material as well as the small amounts of RNA which can be demonstrated around such nuclei.

Sometimes one can find cells or groups of cells which apparently are isolated by strands of nervosa tissue. These cells are not dying, as they closely resemble cells of the adenopituitary. Moreover their connection with neighbouring parts of the adenohypophysis can be detected in serial sections in many instances.

Finally, a number of nuclei can be found near the caudal connection of the pituitary with the tuber cinereum. These nuclei closely resemble those found on the other side of this connection in the brain. Accumulations of RNA surround these nuclei.

Blood vessels are regularly met with in the pars nervosa. Once a blood vessel could be traced which first ran vertically in the connective tissue capsule at the caudal end of the pituitary and which later on penetrated the pars nervosa in rostro-dorsal direction. Here it ramified, the branches remaining amidst the nervosa fibers. Dorsally, above the pars

anterior and between this and the transitional lobe blood vessels can be observed too.

The AF and CHP staining methods according to Gomori reveal an interesting structure. More or less round accumulations of intensely Gomori positive material (neurosecretory substance) are found, which in quite a number of instances are observed to be situated in the nerve fibers. Their dimensions vary from 0.3–2.5 μ. Accumulations larger than 2.5 μ (maximally 18 μ) are rarely found. These larger ones make the impression of being situated between the nerve fibers. They do not always stain evenly, but different shades of purple can be observed after AF. Accumulations can be observed all over the pars nervosa. However, the closer to the caudal connection the larger and (or) the more numerous they are and the closer together they lie. Above the pars anterior, in the strands between this part and the transitional lobe and in the fine strands in the transitional lobe only a few accumulations can be met with.

Neurosecretory substance is also to be observed after Azan staining (red) (occasionally a large accumulation with a blue centre and a red perifery was observed) and after staining with iron hematoxylin (Heidenhain). Phase microscopy of fixed, unstained sections reveals the same structures, but not in every case in which neurosecretory substance could be demonstrated by the AF or CHP staining method.

PAS reveals positive material, localized in similar accumulations as are seen after AF staining. These PAS positive accumulations are found in the dorsal part of the pars nervosa and in the strands of nervosa tissue which penetrate the transitional lobe. The accumulations which are found in these strands can be demonstrated by means of the different solubility of this material from that of the glycoproteins in the transitional lobe: PAS staining after freeze drying without fixation reveals the neurosecretory substance, whereas the glycoproteins appear to have been dissolved out.

So the PAS positive material can be demonstrated in the same area and in similar accumulations as the AF positive neurosecretory substance. However, not in all specimens which contain AF positive neurosecretory substance in the area mentioned PAS positive material can be demonstrated as well.

Pictures are seen which point to a transfer of AF or CHP stainable material from some part of the adenohypophysis into the neurohypophysis, or vice versa: often a distinct track of AF positive material is observed in the caudal but never in the rostral connection. This track extends from this connection into the tuber cinereum in rostral direction, just dorsally of the ependyma. In several specimens it has been observed that, in the tuber cinereum above the pars anterior, such a

track bends in dorsal direction, thus reaching the nucleus praeopticus.

The number and size of the accumulations of neurosecretory substance diminish in the direction just mentioned.

F. EPENDYMA, RECESSUS HYPOPHYSEOS AND CONNECTIVE TISSUE CAPSULE

Finally some remarks on some structures, which are closely related to the pituitary, will be made.

The ependyma, which is situated ventrally of the infundibular cavity, consists of an epithelium of the cylindrical or cuboidal type above the pars anterior and the rostral part of the transitional lobe and of the cuboidal type above the rest of the transitional lobe. The cells possess large nuclei. The protoplasm is slightly stainable. Ventrally of this series of ependymal cells a basement membrane is found, which stains strongly with anilin blue and after PAS.

The ependyma, situated above the caudal part of the transitional lobe and the pars intermedia consists of more rounded cells, lying close together, thus forming a distinct border between the pars nervosa and the cavity. No basement membrane is seen here, whereas some ependymal cells are seen to possess elongations which run in rostral direction just under the layer of ependymal cells.

The ependyma, which lines the IIIrd ventricle (so dorsally of the infundibular cavity) is often squamous. Sometimes, in the least flattened cells, elongations penetrate the tissue of the brain. These elongations bend and run horizontally.

The infundibular side of the rostral connection of the hypophysis with the brain (as visible in a longitudinal section) is covered with cuboidal ependymal cells. So these cells constitute a transition between the ependyma above the pars anterior, if cylindrical, and the squamous cells with which the recessus infundibuli is covered.

The caudal connection is covered at its infundibular surface with cuboidal or squamous ependyma.

The recessus hypophyseos (see fig. 4 and Plate IV 2), which penetrates the pars nervosa medially in dorso-ventral direction at about the caudal third of the longitudinal axis of the pituitary is covered at its beginning with cuboidal ependyma. Later on the recessus ramifies manifold. The finest branches, which are about circular in cross section, can be found in the pars nervosa and, whether or not visibly surrounded by nervosa tissue, in the pars intermedia and the transitional lobe. These branches are no longer covered with epithelium, but with a very thin membrane, the cellular character of which could never be observed.

The connective tissue capsule around the pituitary is composed of two layers. The inner layer is strongly fibrous and stains heavily after Azan staining. Some elongated nuclei of fibroblasts are found here. This layer fits tightly around the pituitary. The outer layer is fibrous as well, but is looser and thicker. Here blood vessels are present.

V. DISCUSSION

A. CELL INCREASE AND DEGENERATION

Juxtanuclear granules (centrioles) have been found with certainty in the acidophilic cells of both the pars anterior and the transitional lobe, in the dorsal lining cells, in the cells of the chromophobic cell area in the pars anterior and in the less chromophilic cells of the pars intermedia. Probably on account of technical difficulties (lack of colour contrast in the two staining methods by which they can be demonstrated) they could not be observed in the basophilic and chromophobic cells of the transitional lobe and the more chromophilic cells of the pars intermedia. Although conditions are favourable for detection of the centriole in the chromophobic cells of the acidophilic cell area in the pars anterior, they have never been observed there.

Nothing seems to be known of the chemical make-up of the centriole (Brachet, 1957) whereas only one communication seems to exist on the chemical composition of the centrosomes. Stich (1954) reports that the centrosomes of the eggs of *Cyclops strenuus* contain basic proteins, RNA and glycoproteins.

In the present investigation the juxtanuclear granules are shown to contain glycoprotein: they are Schiff positive only after periodic acid oxidatione, dimedone is able to block the PAS reaction, saliva does not digest the PAS positive material and their solubility corresponds with that of the glycoprotein of the transitional lobe. Formol fixation is the preferable method to preserve them.

Their possible role in the pituitary of the adult Stickleback is not clear. Not all specimens show juxtanuclear granules, but if they do, they can be present in nearly every cell of e.g., the pars anterior (see Plate III–2), and only one granule per cell is found. A dividing pituirary cell has never been found with certainty (one mitosis has been found, which could have concerned the nucleus of a fibroblast). However, as the material investigated is limited in the sense that only during daytime mainly winter and spring animals were killed, mitosis cannot be completely excluded. Eventual seasonal and daily periodicity can have escaped our observation.

Besides mitosis the possibility of cell division by amitosis should be

considered. Many bean shaped nuclei and nuclei with more or less sharp indentations, even pictures in which the nucleus is almost fully ligatured have been observed (see fig. 5 and Plate III–1). Moreover, in acidophilic cells of the transitional lobe some binucleated cells are observed. These cells were only met in a few instances. This could indicate that cytokinesis immediately follows the karyokinesis and lasts short.

If amitosis is accepted as a possibility for cell multiplication in the pituitary of the Stickleback, then the shape of the nuclei (with indentations) of the following cells makes it likely that these divide directly: the acidophilic cells of the pars anterior, all cells of the transitional lobe and of the pars intermedia.

It is striking that cell multiplication in order to keep on a level or to increase the content of mature cells of the pars anterior seems to happen by amitosis of the mature acidophiles only. The chromophobes both of the acidophilic and of the chromophobic cell area and the dorsal lining cells do not show nuclei with indentations.

As no signs of division can be observed in the dorsal lining cells of the specimens investigated this tissue can hardly be expected to generate new cells in the adult animal. It is the opinion of Bock (1928) that the chromophobic cells originate from the dorsal lining cells. On leaving the epithelial arrangement they should loose their stainability and become chromophobes. During the time they wander to the area of the acidophiles their cytoplasm should become acidophilic. Indeed all kinds of transitions from purple to colourless to red are to be found. Consequently Bock considers the dorsal lining cells to be generative and the acidophiles as adult stadia in the process of growth. Perhaps the best explanation for the occurrence of transitional stadia and for the absence of signs of division is the assumption of a generative function of the dorsal lining cells in the earlier life of the animal. In adult life the chromophobic zone, if present, could be the remnant of such a development whether or not the cells of this zone have a definite function in the adult (see below). Seen in this light the fully acidophilic cell, which is observed lying between dorsal lining cells is no more than a cell developed in the proper direction, which has just failed to displace.

The acidophiles of the transitional lobe seem to duplicate in the same manner as those of the anterior part (see fig. 5 and Plate III–1). If such a duplication should be preceded by release of the secretory products, no binucleated, distinctly yellow acidophiles should be found. Moreover, in this case transitional stadia between colourless and strongly yellow or red acidophiles should be met with in the transitional lobe. But binucleated cells always show a strong acidophilia

and transitional stadia have never been observed. Here too a functionally mature cell divides amitotically.

In the same way again the basophilic cells of the transitional lobe should divide.

All the above mentioned cell types can be regarded as functionally mature on account of their content of stainable specific cell material. The chromophobes of the transitional lobe, contrary to those of the pars anterior, should divide amitotically whether they are functionally mature or immature. Because of the many transitional forms between true basophiles and true chromophobes a secretory cycle is accepted here, in which both play a role. How far the chromophobes of the transitional lobe contain γ-cells in the sense of ROMEIS (1940) or can exhibit γ-cell function is not settled for the Stickleback.

Once again, in the pars intermedia, one cell type which can be regarded as functionally mature on account of its contents of basophilic PAS positive material and one cell type of which the functional maturity can be doubted, seem, on the ground of the presence of nuclei with indentations, to be able to divide amitotically in the adult animal. Just as in the above mentioned basophilic and chromophobic cells a secretory cyclus is accepted for the more and less chromophilic cells of the pars intermedia on account of the transitional forms between both cell types.

If the cells of the pituitary are able to divide in this way, increasing the cell number, one could expect to find degenerating cells as well. Cells of the latter kind have been observed in the pars nervosa, which are not very numerous, just as BOCK (1928) has described, and in the pars anterior. In this respect the basophilic PAS positive bodies should be mentioned, a few of which show clearly a structure like the Hassall bodies in the thymus: small oblong cells are deposited against such a body.

B. CELL TYPES

ROMEIS (1940) on account of his results with the Azan and Kresazan methods distinguished 7 different cell types in the pars anterior generally of human material: the undifferentiated cell, the α-, β-, γ-, δ-, ε-cell, and the degranulated vacuolated cell.

If we do not attach a great value to the name "pars anterior" in fishes, which very probably has not been applied after an extensive investigation on possible homology with that part in mammals, the cell types found in the pars anterior together with the transitional lobe can be compared with those of the pars anterior in mammals.

As a result of Azan staining the acidophilic cells of the pars anterior can be regarded as similar with the α-cells of ROMEIS.

The acidophilic cells of the transitional lobe resemble the ε-cells of the mammalian pars anterior. The possibility that the acidophiles of the transitional lobe stain yellow or red agrees with the properties of the ε-cells. Romeis explains this staining difference by stating that large granules have a greater affinity for azocarmine, small granules a greater affinity for orange G. This explanation is not confirmed by our staining results as the greatest granules observed can take a yellow colour.

Both the basophilic β- and δ-cell of Romeis should be represented by the basophilic cells in the Stickleback's pituitary. Romeis has differentiated between both cell types on account of his Kresazan staining. This staining is not applied in this investigation, but, as will be seen below, no differentiation within the group of the basophiles in this animal could be made as a result of the PAS and AF staining methods.

The remaining three cell types of Romeis, the undifferentiated cell, the γ-cell, and the degranulated vacuolated cell, should then be represented, if present, by the chromophobic cells of the pars anterior and the transitional lobe. The chromophobes of the chromophobic cell area in the pars anterior can be regarded as undifferentiated cells. They can be present in fairly large numbers in the adult animal and do not seem to develop into acidophiles very readily. The assumption that they are undifferentiated cells fits in with their assumed development from the dorsal lining cells in earlier life. On the other hand these cells are not likely to be degranulated acidophilic cells, as their localization in this respect is not reasonable. Whether these cells can be regarded as undifferentiated cells or have a proper function (γ-cells of Romeis) cannot be settled by the results of this investigation. At least part of the chromophobes of the acidophilic cell area could probably be similar to the degranulated vacuolated cells of Romeis, although in our material no vacuolation is observed. An observation which points in this direction is that of the exceptional pituitary (see p. 168) in which distinctly many transitions between acidophiles and fully degranulated cells are observed.

The results reported here do not exclude the presence of undifferentiated cells and properly functioning γ-cells for the chromophobic cells of the transitional lobe. As stated above at least part of the chromophobes are regarded as degranulated basophiles. In mammalian tissue this cell type is vacuolated (cf. Romeis, 1940). In our material only in a few cells vacuoles could be observed.

The dorsal lining cells resemble those cells of the mammalian pituitary, which are found dorsally in their pituitary. Their granular content and their abundance in RNA make a proper secretory function very likely.

In the above comparison with the pars anterior in mammalian pituitaries the transitional lobe is treated together with the pars anterior. That it is tempting to implicate the pars intermedia in this comparison on account of its staining behaviour will be seen later on.

C. SECRETORY PRODUCT

In most sections of the usual thickness the cytoplasm of the acidophilic cells in the pars anterior stains homogeneously red. Only a small quantity of this acidophilic material is clearly seen to be located in granules (up to 0.8 μ, in one exceptional case up to 1.5 μ). It is accepted, however, that this material is stored in granules to a greater extent than is visible. For this a number of reasons can be advanced:

1. an occasional thin offshoot of such a cell can show a distinct single layer of granules,

2. one μ thick sections show granules in a greater number of cells than 5 μ thick sections,

3. RINEHART and FARQUHAR (1953) from electron microscopic investigations describe the acidophilic material as localized in granules (in the order of 0.35 μ, in rat pituitaries). In connection with these findings the observations described under 1. and 2. indicate that, at least for the greater part, the acidophilic cell substances in the pars anterior of the Stickleback are localized in granules as well. However, the dense storage hides the separate granules from observation in most sections used for light microscopy. This opinion does not exclude, that another part of the acidophilic material, e.g., that which is going to be released from the cell, could be in a dissolved state.

The question, whether the acidophilic substances are stored in granules or not could probably be solved by cutting very thin sections and using the electron microscope. The same problem could thus be solved for the acidophiles of the transitional lobe, the maximal dimensions of the granules are here somewhat smaller than in the anterior lobe (about 0.3 μ).

The dorsal lining cells contain their specific amphoteric cell product in granules mainly, but some clumps of small size can be observed as well. Here the cells are not densely filled and nearly all granules can be seen separately.

Among the secretory material of the basophiles, which appeared to be of glycoprotein nature, many distinctly defined granules can be seen. Besides this, clumps of variable shapes and dimensions are observed. Here again the question could be raised whether these clumps are composed of a number of granules, which are crowded together and consequently are not separately visible.

One of the main differences between formol liquid fixation and freeze drying followed by formolgas fixation, observed in the transitional lobe is the fact that with the latter technique somewhat more and sharper defined granules are observed. This better spatial cytochemical localization may have been brought about by the rapid solidification by quenching and the subsequent fixation, which is adequate for glycoproteins.

In the pars intermedia the picture of localization of the glycoproteins is much more satisfactory after quenching in Freon 13 (chilled with liquid air, -180° C) and freeze drying followed by formolgas fixation. These glycoproteins can be demonstrated as localized in fine granules.

The neurosecretory substance which is found in the pars nervosa and which stains with AF and CHP is partly localized in small granules which sometimes could be observed within nervous fibers. Sometimes threads of similarly stainable material are seen within these fibers. Other, larger accumulations, with the shape of Herring bodies, are found between the nerve fibers. These Herring bodies can show an aberrant staining behaviour. After Azan they may turn out to be blue with a red periphery. Whether the PAS positive material which is found in part of the pars nervosa is identical with the neurosecretory substance, in other words whether the neurosecretory substance contains a carbohydrate component, is not yet fully established.

That the transitional lobe (German: Übergangsteil) is really a transition between the pars anterior and the pars intermedia must be doubted. The name has been given by STENDELL (1914) on account of the penetration of acidophilic cells from the pars anterior and of the penetration of strands of nervosa tissue; the transitional lobe thus has characters in common with both the pars anterior and the pars intermedia (also DE BEER, 1926). Although the acidophilic cells of the pars anterior and the transitional lobe have characters in common (their specific cell product showing acidophilia, the occurrence of juxtanuclear granules and the presence of RNA accumulations) there are at least three differences which point to the functional divergence between these cells. These differences are their possible staining differences (yellow-red), the absence of tinctorially transitional stadia (from pars anterior acidophiles to transitional lobe acidophiles) (if the acidophiles of the transitional lobe are stained differently from those of the pars anterior in the same animal) and the maximal diameter of the granules, 0.8 μ in the pars anterior and 0.2–0.3 μ in the transitional lobe. STENDELL's statement (1914) is mainly based upon investigations of *Anguilla vulgaris*, *Mormyrus caschive*, *Cyprinus carpio* and *Esox lucius*, where a gradual transition of the acidophiles of the pars

anterior to those of the transitional lobe is said to occur; it does not apply, however, to the Stickleback.

The secretory products are believed to be at least partly, transported from the pituitary by the blood. Blood vessels are met in every part. This is in accordance with the finding of STENDELL (1914), who describes a very distinct blood supply in *Anguilla*, *Cyprinus*, *Mugil*, *Chrysophys*, *Salmo*, *Gadus*, and *Mormyrus*; for *Esox* an extremely rich blood supply is mentioned. It is, however, in contrast to the observation of BOCK (1928) who states to have met no or almost no blood vessels in sections of the pars anterior of *Gasterosteus aculeatus* forma *leiura*. Only in the pars intermedia the blood supply is comparatively scarce.

The secretory products of the basophiles and of the more chromophilic cells of the pars intermedia have been demonstrated to be clycoproteins. Although, generally speaking, strong PAS positivity corresponds with strong anilin blue staining and faint PAS positivity with faint anilin blue staining, other correspondences are realized as well. If it is accepted that the anilin blue stained material is a precursor substance (BOCK, 1928), whereas the material which is stained by PAS is the end product, then the above mentioned different interrelations can be brought about by differences in the rate of synthesis, storage and release.

Analogous to what is found in mammalian pituitaries it can be thought that the glycoproteins represent thyrotrophin as well as one or both gonadotrophins in the Stickleback. If we accept that the AF staining method reveals the thyrotrophic hormone in the Stickleback pituitary, just as it does in the β-cells of the rat pituitary (HALMI 1950, 1951, 1952a, 1952b; PURVES and GRIESBACH, 1951a, 1951b, 1951c), then the thyrotrophes should be localized in the transitional lobe.

As, however, on the grounds of the present study no choice can be made between the possibility of production of two different substances in the basophiles of the transitional lobe and the possibility of production of one substance stainable with both PAS and AF, it cannot be excluded that gonadotrophin is produced in this pituitary part as well. If so, it is probably LH (PICKFORD and ATZ, 1957).

To this it can be added that the methyl blue staining behaviour of the glycoprotein in the transitional lobe is in accordance with that of LH in the δ-cells of the rat pituitary (WILSON and EZRIN, 1954; RENNELS, 1957; HILDEBRAND, RENNELS and FINERTY, 1957): it remains red in the PAS methyl blue technique.

PAS positive material, which turns purple upon methyl blue staining just as FSH does in the δ-cells of the rat pituitary, is found in the pars intermedia of the Stickleback's hypophysis. If it is permissible to apply to the Stickleback what has been found in the rat, this material should be FSH.

Above no explanation is given for the occasional presence of a dorsal part of the transitional lobe, the significance of which is not at all clear.

It must be borne in mind that the correlations of stainable materials with certain pituitary hormones is fully speculative, as no proofs can be given here. Still, these considerations could be a starting point for further investigation, in which histological and histochemical changes in the pituitary cells brought about by adequate experimental conditions could help to elucidate the histo-endocrinological organisation of the pituitary of the Stickleback.

VI. SUMMARY

1. With the object of this investigation, the pituitary of the Three spined Stickleback *(Gasterosteus aculeatus* L. forma *trachura* Cuv.*)* a fixation procedure is worked out in which the frozen dried piece of tissue is kept dry during fixation in gaseous formaldehyde. This procedure has given better staining results (Azan, PAS, AF and CHP) than the conventional procedure in this case freeze drying followed by fixation in absolute alcohol or acetone. It seems likely that the readily soluble cell substances dissolve out of the tissue in the conventional procedure. Dissolution or dislocation of these cell substances can be prevented by the use of a non-liquid in this case gaseous fixative.

2. Fixation in gaseous formaldehyde can also be applied to deparaffinized or undeparaffinized tissue sections, which are obtained after freeze drying, unfixed embedding in paraffin and sectioning.

3. Freeze drying followed by formolgas fixation reveals the glycoproteins in a better localization compared with formol liquid fixation (without freeze drying): sharper borders of granules and clumps have been observed.

4. The situation of the pituitary in the brain cavity and the histology of the four parts of the pituitary: the pars anterior, the transitional lobe, the pars intermedia (these three parts compose the adenohypophysis) and the pars nervosa (neurohypophysis) is described.

5. On account of the Azan staining results the different cell types, which can be found in the various adenohypophysial parts, are described. These cell types are:

in the pars anterior: the acidophilic cells, the chromophobic cells found in the acidophilic cell area, the chromophobic cells of the chromophobic cell area, and the dorsal lining cells,

in the transitional lobe: the acidophilic cells, the basophilic cells, and the chromophobic cells,

in the pars intermedia: the more and the less chromophilic cells. The agreement on account of the Azan staining behaviour of the different

cell types in the pituitary of the Stickleback with the cell types described by ROMEIS (1940) is discussed.

6. For all these cell types the secretory material, juxtanuclear granules and RNA, if present, is discussed.

7. The juxtanuclear granules (centrioles) appear to contain glycoprotein.

8. Abundant amounts of RNA are observed in the cells of the pars intermedia and in the dorsal lining cells. Little RNA is found in the chromophobic cells of the pars anterior and in some cells of the transitional lobe. Moderate amounts are found in the other cells.

9. The PAS positivity in the cells of the transitional lobe and the pars intermedia, which appears to be due to glycoproteins, is positively correlated to the results of the anilin blue staining in the Azan method.

10. In an attempt to correlate the localization of the AF positive material with the localization of the PAS positive material in the transitional lobe it appears that, with a few exceptions, the localization of the granules and clumps is the same after both staining methods. From these observations it remains uncertain whether two different substances are produced in the basophiles of the transitional lobe, of which the one is AF positive and PAS positive and the other is PAS positive only, or one substance, which stains with both PAS and AF, is produced.

11. In some specimens a dorsal zone in the transitional lobe is observed after Azan and PAS staining. This zone could be distinguished from the rest of the transitional lobe on account of the shape and the number of PAS positive granules and clumps.

12. Some particulars about the pars nervosa, the ependyma, the recessus hypophyseos, the blood supply and the connective tissue capsule are given.

13. The occurrence of PAS positive material which is localized in the same area and in similar accumulations as the AF positive neurosecretory substance is mentioned.

14. A distinct difference in the microscopical anatomy of the pituitary of the forma *trachura* and the forma *leiura* is found in this sense, that in the pituitaries investigated of the forma *leiura* a definite arrangement of acidophilic cells of the pars anterior around the blood vessels is met with, whereas in the forma *trachura* this is not found.

15. Besides mitosis amitosis is considered as a possibility of cell multiplication on account of the occurrence of nuclei with more or less deep indentations.

16. Signs of cell degeneration are observed in the pars nervosa. In this light the basophilic bodies of the pars anterior are considered as well.

17. The dorsal lining cells are considered to play no role in the production of cells in the adult life of the animal.

18. The question, whether the production of TSH, LH and FSH, if present, is localized in three different cell types needs further investigation.

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AN ATTEMPT AT CLARIFYING SOME CONTROVERSIAL NOTIONS IN ANIMAL PSYCHOLOGY AND ETHOLOGY

by

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AIM OF THIS PAPER

Recently, in a long "letter to the Editor" of the British Journal of Animal Behaviour, HALDANE (1956) corrected some misconceptions found among ethological authors with regard to the history of certain technical notions current in the scientific study of instinct. He pointed out that these notions and some mechanical "models" illustrating them were devised long before present day ethology emerged (e.g. by McDOUGALL, SHERRINGTON and H. JACKSON), and he discussed some aspects of them. Since, however, HALDANE dealt particularly with the *origin* of these notions he stressed the points of agreement between authors and ignored the disagreements and controversies between them. Consequently his account tends to conceal some essential divergences and thus his account is apt to lead to confusion. An attempt will therefore be made in this paper to clarify some points which are relevant to Haldane's discussion. My theme deals particularly with the distinctions between apparently identical and homonymous notions in different conceptual systems, and it will be approached by drawing some broad lines from a historical point of view. For reasons of space no consideration will be given to the contributions made by medical and physiological psychology and neurophysiology with regard to the items to be considered.

[1] The writer wishes to express his thanks to Miss E. M. BARRAUD of Cambridge for her help in correcting the English text. He is indebted to Prof. LORENZ for reading the MS and making critical comments which have resulted in a better mutual understanding, in spite of certain existing disagreements. This does not imply, of course, that Prof. LORENZ is in any way responsible for the account of his ideas given in this paper.

FROM CHAIN REFLEXES TO GOAL-STEERED BEHAVIOUR

In the earlier decades of this century the then current concepts of instinct held by most biologists and animal psychologists could still largely be defined as one or other type of chain-reflex, a unit consisting of an innate stimulus and an innate response linked up by an innate connection. This concept may be indicated by a formula: The starting or *primal* stimulus, to be represented by PS, causes a reaction R, which results in a new stimulus situation to be represented by QS, in which the primal stimulus is removed or made ineffective. Thus in the new situation the cause of unrest is *quiesced*, as indicated by the sign *o* (= *nullification*):

$$\begin{tikzcd} \text{PS} \arrow[r] & \text{R} \arrow[r] & \text{QS} \arrow[ll, bend right, dotted, "o"'] \end{tikzcd}$$

In the field of classical animal psychology it was W. McDougall (1908, 1923), W. Craig (1918) and E. C. Tolman (1932) in particular who criticised this too simple chain-reflex scheme and added two other essential attributes to the then concept of instinct: Firstly, the notion of "*appetite*" or instinct-specific intrinsic "*energy*", here to be represented by E, which causes "fluctuations" or "cycles" (Craig), or "spontaneity of behaviour" (McDougall), or "physiological states of agitation" (Tolman), and which gives rise to a variety of appetitive actions, AA_1, AA_2, AA_3 etc., which all result in one and the same end. (Not *re*-actions, since the behaviour is now conceived to be "spontaneous".) And, secondly, the notion of *consummation* or *goal*, G, in which all these appetitive activities result and the attainment of which brings about the discharge *and/or* "quiescing" (Craig, Tolman), or "satisfaction" (Craig, McDougall), or nullification, *o*, of the particular appetite or energy concerned. In other words, the attainment of the goal makes the train of activities stop by *removing* its *driving* energy. The core of the principle was formulated by McDougall as early as 1908 as follows: "All instinctive behaviour exhibits a persistent striving towards the natural end of the process. That is to say, the process is not to be arrested by any sufficient mechanical obstacle, but is rather intensified by any such obstacle and only comes to an end either when its appropriate goal is achieved, or when some stronger incompatible tendency is excited, or when the creature is exhausted by its persistent efforts". (Loc. cit., p. 27.) Since, in the light of this concept, the appetite or energy may also activate the behaviour if no proper primal stimulus is present (in contrast with reflex behaviour), the PS in the original scheme must now be put between brackets, and thus the new formula becomes:

$$\begin{tikzcd} & & O & \\ & & AA_1 \arrow[dr] & \\ (PS) \arrow[r] & E \arrow[ur] \arrow[r] \arrow[dr] & AA_2 \arrow[r] & G \\ & & AA_3 \arrow[ur] & \end{tikzcd}$$

In order to prevent misunderstanding, we must at once make clear that the notion of "goal" or "end" or "purpose" as conceived by the authors cited, has *nothing to do with* what is often rather awkwardly called the "biological function" or "teleological purpose" or "functional adaptation" of behaviour instead of being accurately termed the "survival value". Since the term "survival value" indicates exactly what it means, there is no reason to confuse the strictly *behavioural* notion of "goal", "end" or "purpose" as applied in motivation and steering theory, with the strictly *ecological* notion of "survival value" as used in evolution theory. For example, the patella-reflex is neither a goal-steered activity nor is its performance a goal in itself, although it definitely has a survival value. Conversely, smoking is a goal keenly pursued by many people, although it is definitely detrimental to survival.

In behaviour theory, a goal may therefore be defined as: an action or situation which causes the ending of a molar behaviour sequence which is controlled by a system of cybernetical mechanisms reacting upon variable environmental and homeostatic stimuli and working *in such a way that*, normally, the *choice* between different molar behaviour alternatives (including the starting and stopping of behaviour) *will tend to result in* this particular end-activity or end-situation. (In previous papers I gave what I hoped were more understandable definitions, but they appear to have evoked misunderstanding! One could, however, simply say that the behaviour is steered by its result towards its environmental or homeostatic goal.) Clearly, this notion has in itself nothing to do with the notion of survival value. And further, it should also be stressed that "goalism" and "wholism" are entirely different things.

For the rest, however, the behavioural notion of "goal", even when applied in this strict sense, still has a two-fold meaning: Firstly, it may indicate the phenomenon that a certain train of activities may show *variations* in *space* and *pattern* which are characterised by the fact that they tend to result in an invariable end; and secondly the notion of goal may indicate the phenomenon that a certain invariable train of activities will *continue until* a certain change or event is effectuated. Usually, in descriptions of appetitive behaviour, these two criteria are combined (see, e.g., McDougall's "Seven Marks of Behaviour", Nos. 3 and 4, 1923), and sometimes they are even mixed up, but in some cases it may be very useful to distinguish between them. Clearly they are based upon different types of feedback mechanisms. (In particular, the former type is entirely different from the extremely simple "thermostat type" of feedback mechanisms usually studied by physiologists and neurologists.)

We may distinguish, therefore, between—on the one hand—appetitive behaviour which is variable in direction, pattern, intensity and duration, and—on the other hand—appetitive behaviour which is variable *only* in intensity and duration. The two types have in common that, in both combinations of notions, the "goal" is assumed to be the cause which stops, and which alone can stop, the train of the "appetitive behaviour", apart from force majeure.

Further, it must be made clear that a "molar behaviour sequence" in the above definition may be temporarily interrupted by other activities without thereby losing its character of being *one single* molar behaviour sequence. For example, nest-building may be regularly interrupted by eating, sleeping etc., but nevertheless we are justified in saying (at least in some species) that it *continues persistently* for days or weeks until it is stopped by its environmental goal, viz. the presence of a complete nest, i.e. a structure of certain dimensions and solidity, provided with a horizontal rim, etc. So the appetitive behaviour of nest-building must be regarded as one single sequence of activities steered by its result towards its goal, provided that there is sufficient evidence to prove that it is indeed controlled by such mechanisms as mentioned in the definition. Similarly, amorous courting in certain birds may be said to be *one single* molar sequence of variable appetitive behaviour since it will go on persistently for days or weeks until it is stopped as a result of the formation of a marital bond. And in the same way, the fighting in certain gregarious mammals (e.g. cows) may be considered to be *one single* sequence of appetitive behaviour aiming at a stable social organisation, firstly because this behaviour is variable (dominance versus submission) and secondly because it will persist for days or weeks until such a social organisation has become definitively established, after which it will stop. Quite recently, in a critical discussion of the concept of goal as I defined it in 1940–1955, HINDE (1957, p. 117, point IV and footnote) has fundamentally misinterpreted this aspect of the concept, and it must therefore be stressed again here.

Up to this point this account has been formulated in such a way as to cover not only the concept of instinct developed by the authors quoted, but also the one developed since then by LORENZ and his adherents (to be discussed later) and to include also the "psycho-pneumatic" or "psychohydraulic" models sketched by McDOUGALL (1908, p. 44; 1913, pp. 861–872; 1923, pp. 106–109, and also 171–172 and 218–219!) and LORENZ (especially 1950, pp. 255–257, and 1952, pp. 39–40) which were the subject of HALDANE's paper. It will be clear, therefore, that with regard to the points considered so far, *all* these authors are essentially in line with one another. Such notions as, for example, instinct-specific threshold fluctuations and instinct-specific nervous exhaustion are implicit in these concepts (see, e.g., CRAIG, 1918, p. 102, and McDOUGALL, 1923, p. 108), and they were elaborated by several followers of McDOUGALL, e.g. by PORTIELJE (1928) in his concept of "instinctive object transference".

Moreover, since both McDOUGALL (1923) and TOLMAN (1932) explicitly stated that they had largely derived their ideas on appetites, nervous energies and quiescing agencies from CRAIG (1918), and since related ideas were developed by other authors who need not be mentioned here, and further considering that LORENZ (especially in

his earlier writings) often entered into discussion with these classical authors, we may safely assume that all these ideas as well as their mechanical analogies go back as early as the epoch when "animal psychology" and "ethology" were still undifferentiated, except that LORENZ's concept was also influenced by the notion of "Leerlaufen" originally coined by HELLMAN (as LORENZ informs me) and introduced in scientific literature by HEINROTH (1910). In these matters there is indeed a straight path of development from MCDOUGALL (1908) via CRAIG to MCDOUGALL (1923) and TOLMAN (1932). One has only to glance through the Journal of comparative and physiological Psychology to recognize the influence of this trend in American animal psychology. (See also the reformulation given by FRENCH, 1952.) From this path was the branch developed by LORENZ. (Of course MCDOUGALL and CRAIG too had their predecessors, and moreover FREUD developed similar ideas in his neurochemical theory on the libido, but these other authors are not to the point here.) To be sure, TINBERGEN (1951, p. 105) stated that CRAIG's paper "has not received the attention it deserves", and similar statements may often be found elsewhere in ethological literature, but nevertheless CRAIG's notions of appetite and quiescing formed the basis of the motivation theories subsequently built up by MCDOUGALL, TOLMAN and LORENZ, bien étonnés de se trouver ensemble. (See, e.g., MCDOUGALL, 1923, Chapters IV–V, and TOLMAN, 1932, Chapters XVIII–XX.)

THE BASIC TYPES OF "GOALS", OR QUIESCING AGENCIES

The divergence comes in, however, as soon as one tries to define the meaning of G in the above formula, that is if one attempts precisely to state which agent or mechanism accomplishes the discharge *and/or* quiescing, or satisfaction, or consummation of the drive, or appetite, or energy or whatever it may be called. There is much confusion on this point and, since HALDANE's account is liable to evoke further misunderstanding, this item must now be considered.

One source of confusion may have a purely linguistic origin. Personally I often received the impression that many continental European ethologists in some way or other derive the term "consummatory" from the verb "to consume" and that consequently they are inclined to think that a "consummatory act" is an act which consumes nervous energy, and this idea would then at the same time imply that a "consummatory stimulus" is nonsense. Such a linguistic misunderstanding is at least invited (if not expressed) in the LORENZ-TINBERGEN definition according to which a consummatory act is an act which "consumes" the nervous energy (LORENZ, 1950, p. 248;

1952, p. 38; Tinbergen, 1951, p. 105). To many British and American readers this linguistic misunderstanding may seem silly, but I am inclined to think that at least some continental European colleagues will at this point be reaching for their dictionaries!

Secondly, many present day ethologists seem not to be aware that the terms "appeted stimulus", "consummatory stimulus" and "consummatory action" were employed by Craig in a rather vague and ambiguous sense which included much more than the more sharply defined technical terms "releasing stimulus" and "consummatory act" used today. It is indeed curious to see how McDougall and Tolman on the one hand, and Lorenz on the other hand, derived from Craig's exposition exactly opposite concepts with regard to the discharging and/or satisfying agency! It is well worth considering these points in greater detail. Here again many modern ethologists who do quote, but appear to misinterpret Craig's papers, may be surprised when confronted with what seems to be the actual situation.

As a result of careful reading, it seems justifiable to distinguish in Craig's description the following *types of "consummatory" phenomena:*

a. The "incipient consummatory actions" which usually precede and often accompany the appetitive behaviour, and which are identical with what were called "intention movements" by Heinroth. With regard to *these* activities the term "consummatory" is, strictly speaking, out of place and therefore they need not be considered further.

b. The "consummatory stimuli" or "consummatory situations" which are attained as a result of appetitive and/or aversive behaviour and which are *in themselves* quiescing (i.e. satisfying) agencies, without the intermediacy of, and without being followed by, any energy-discharging consummatory action. ("When he has got rid of the disturbing sight, his agitation ceases"; loc. cit., p. 100; see also pp. 91 and 94.) This type of quiescing resulting from a mere change of stimulus situation is characteristic of fleeing, fighting and sleeping behaviour, for Craig regarded the end-actions of fleeing and fighting as aversive (i.e. *negative appetitive*) behaviour and he did not consider sleeping to be an action. (Loc. cit., pp. 92, 93–94, 100, 101.) This type of quiescing process may now be termed "Type 1" and, representing the quiescing stimulus by QS, its formula may be rendered:

$$\begin{tikzcd}
 & & O \arrow[ddll, dotted] \arrow[ddl, dotted] \\
 & & AA_1 \arrow[dr] \\
PS \arrow[r] & E \arrow[ur] \arrow[r] \arrow[dr] & AA_2 \arrow[r] & \mathbf{QS} \\
 & & AA_3 \arrow[ur]
\end{tikzcd} \qquad \text{(Type I)}$$

Clearly, this Type I of quiescing processes includes a very wide range of behaviour phenomena. On the one hand, under it may be classified such simple patterns as orientation movements, taxis components and similar minor patterns with regard to which it might be doubted whether they should be regarded as appetitive behaviour or as reflex behaviour of the PS → R → QS type. On the other hand, under Type I might be classified such highly complicated and variable major behaviour systems as, for example, nesting in those species in which nest-building persists until the nest is finished and will always stop as soon as the nest is finished; or pair-formation in those species in which the bond-forming display goes on until the marital bond is formed and then stops (in those species in which this display is not correlated with the copulatory behaviour), and similar major systems of behaviour. From Craig's account it is not clear how far he extended his concept of the "consummatory stimulus", and this circumstance is responsible for much of the confusion and controversy which will be discussed further on in this paper.

c. The "consummatory stimuli" or "objects" which are *not* in themselves quiescing (satisfying) agencies but which "stimulate" (i.e. *release*) a "consummatory action" which in its turn, by being performed, quiesces (satisfies) the appetite *as a result of* the motor discharge of previously accumulated energy, and which action may therefore be called the quiescing act, or QA. In this type of consummation (which will be called Type II) it follows implicitly that the action can go on even if the releasing stimulus, RS, disappears during the consummatory performance. Consequently we may distinguish here between the *primary* goal, i.e. the invariable end of variable appetitive behaviour, namely the RS, and the *ultimate* goal, i.e. the quiescing discharge of motor energy by means of the QA. The formula is:

$$\begin{tikzcd}[row sep=small] & & \mathrm{AA}_1 \arrow[dr] & & \\ (\mathrm{PS}) \arrow[r, dashed] & \mathrm{E} \arrow[ur] \arrow[r] \arrow[dr] & \mathrm{AA}_2 \arrow[r] & \mathrm{RS} \arrow[r] & \mathbf{QA} \\ & & \mathrm{AA}_3 \arrow[ur] & & \end{tikzcd} \qquad \text{(Type II)}$$

Parenthetically, Craig's account does not make it clear whether he has indeed recognised this latter type, although his description of sexual behaviour may be regarded as suggestive of it. ("The stimulation required for discharging the sexual reflex", loc. cit., p. 100.) Surely Lorenz (1950, p. 228) was wrong when he asserted that Craig showed "with a wealth of observational evidence, that the animal as a subject is certainly not aiming at the survival value of its activities, but *merely* at the *discharge* of certain actions." (First italics mine, second italics Lorenz' —A.K.) On the contrary, we may seriously doubt whether Craig ever intended the interpretation of his findings as usually given by Lorenz and his adherents, and actually there is more reason to attribute the discovery of Type II of quiescing to Lorenz himself. (*Postscript:* After reading this paper in MS Lorenz wrote me that, in the quoted statement and in similar ones he had made earlier, he did not intend to go so far as to assert that no other type of quiescing occurs at all. We shall return to this point later.)

d. Quiescing (satisfaction) is attained as a result of a combination of a "consummatory stimulus" or "object" together with a "consum-

matory action" in such a way that the consummatory action does not quiesce, or even cannot be performed at all, if it is not *simultaneously* accompanied by the consummatory stimulus. ("He abandons himself to an orgy, rioting in the wealth of new, luxurious stimuli", loc. cit., p. 98.) In this type of consummation the action and the stimulus are inseparable; and further, the stimulus is at once releasing-and-quiescing. There is no distinction between a primary and an ultimate goal. According to CRAIG's statements this type is the most frequently occurring one. (Eating, drinking, nesting, brooding etc.) Its formula is:

$$
\begin{tikzcd}[row sep=small]
 & & O & \\
 & & AA_1 \arrow[dr] & \\
(PS) \arrow[r] & E \arrow[ur] \arrow[r] \arrow[dr] & AA_2 \arrow[r] & \mathbf{QSA} \\
 & & AA_3 \arrow[ur] &
\end{tikzcd}
\qquad \text{(Type III)}
$$

Up to here, CRAIG's account has been followed. One could, however, envisage the possibility that yet another type exists which is not represented in CRAIG's descriptions but which would logically fit into his broad usage of the term "consummatory": namely, a behaviour sequence consisting of an "appeted" non-quiescing "consummatory stimulus" which releases a *non-discharging* and *non-quiescing* stereotyped "consummatory action" which in its turn results in a quiescing "consummatory stimulus". In this hypothetical type the *apparent* consummatory action would *in reality* be a stereotyped terminal act of the appetitive behaviour, TAA, and consequently this type of end mechanism would represent only a variant on Type I, according to the formula:

$$
\begin{tikzcd}[row sep=small]
 & & O & & & \\
 & & AA_1 \arrow[dr] & & & \\
PS \arrow[r] & E \arrow[ur] \arrow[r] \arrow[dr] & AA_2 \arrow[r] & RS \arrow[r] & TAA \arrow[r] & \mathbf{QS} \\
 & & AA_3 \arrow[ur] & & &
\end{tikzcd}
\qquad \text{(Type Ia)}
$$

This latter type of quiescing is especially interesting because it makes obvious the distinction between the two notions of "goal" indicated earlier. If the "appetitive behaviour" were defined as the behaviour which is variable in pattern and duration, while the "goal" were defined as the invariable end following on the variable phase, then clearly this would imply that the "appetitive behaviour" would embrace exclusively the AA_1, AA_2, and AA_3 activities, while the "goal" would consist of the RS → TAA → QS sequence. If, on the contrary, the "appetitive behaviour" were defined as the behaviour which will go on until a certain change or event is effectuated (i.e. irrespective of whether this behaviour is variable *only* in duration or *also* in pattern), and if the "goal" were defined as the quiescing agency which alone is

able to stop the train of activities by nullifying the driving energy, then clearly this would imply that the "appetitive behaviour" would consist of the whole AA → RS → TAA sequence, while the "goal" would embrace only the QS stimulus. It therefore makes good sense to distinguish, in the last formula, between the "primary" goal, RS, and the "ultimate" goal, QS, and to assign a special symbol to the stereotyped appetitive act that separates these two goals in the time sequence.

The types of quiescing processes outlined above are the basic ones required for the considerations which follow. We shall see later that all these types do really occur in animal behaviour. The formulas are intended to facilitate understanding of the different concepts. These formulas are, of course, a crude simplification of the postulated processes involved. For example, no detailed allowance is made in them for the cybernetical mechanisms by which the animal's "choice" between the different alternatives provided in the AA behaviour is determined in such a way that it is guided towards its primary goal. The formulas would have become too complicated if these steering feedbacks had been inserted in them.

It goes without saying that one may easily envisage even more complicated formulas by extending the number of elements linked in series-connection. For example, by analogy of Type Ia, one might devise similar types IIa and IIIa. Or one might insert in the AA behaviour the locomotory Erbkoordinationen which are separate quiescing mechanisms on their own account. Or one might split up the E into a motor and a sensory component, ME and SE, according to the investigations of Prechtl and others. Or one might distinguish an innate and a learned component in the primal stimulus, IPS and LPS. All this would, however, lie beyond the scope of this critical review which aims at a clarification of certain controversial concepts and at clearing up certain existing confusions.

CONTROVERSY ON THE BASIC TYPES, AND FURTHER IMPLICATIONS

Having analysed the different types of G conceived or conceivable in Craig's description, we are now able to investigate further how these elements were elaborated in the conceptual systems devised by McDougall, Tolman and Lorenz. Since all these conceptual systems have in common the PS → E → AA part of the formula, we need not be surprised that *in this respect* the mechanical models illustrating them are identical, as has been expounded by Haldane. And similarly we need not be surprised that the keys and locks and valves in these mechanical analogies are identical too, because a further common feature in all these systems is that the primary goal at which the appetitive behaviour is directed is always thought to be a stimulus, be it an RS, or a QS, or the S component in a QSA act.

The differences come in, however, as soon as one considers the nature of the "appeted" stimuli (whether RS and/or QS or S component) and the nature of the *ultimate* goal, the quiescing agency (whether QS, or QA, or QSA).

In McDougall's conceptual system there were, at least explicitly, no satisfying agencies recognised other than the types 1 and 1a, i.e. the QS types. "An instinct is to be defined and recognized, not by the kind of movements in which it finds expression, but by the kind of change of the animal's situation which its movements, whatever they be, tend to bring about and which, when it is achieved, brings the train of behaviour to a close. Unlike reflex action, instinctive action strives toward a goal, a change of situation of a particular kind, which alone can satisfy the impulse and allay the appetite and unrest of the organism. We must, therefore, define any instinct by the nature of the goal, the type of situation, that it seeks or tends to bring about, as well as by the type of situation or object that brings it into activity." (Loc. cit., pp. 118–119.) By this definition, McDougall stated *all* motor activities to be essentially appetitive behaviour, whereas *all* quiescing was assumed to be caused by environmental and homeostatic feedback stimuli. For example, food-seeking and eating was conceived to be the appetitive behaviour leading to the quiescing stimulus of a full stomach; nest-building was assumed to be the AA behaviour resulting in the QS stimulus of owning a completed nest; social aspiration and submission were thought together to constitute the appetitive behaviour by means of which the animal aims at the quiescing stimulus of being accepted into a stable, well-ordered social organization; and so on. One might say that McDougall has extended the range of the concept of the "consummatory stimulus" (Type 1) to its furthest limit compatible with the range of the behavioural evidence; and at the same time he made (at least in theory) this type of superordinate goal-steering agencies the *exclusive* corner-stone of his conceptual system. In his further account he was, however, not quite consistent, and actually he did recognise the QSA type with regard to mating, brooding and perhaps sleeping. For the rest he made clear that, in his concept, the criterion from which a "goal" is inferred is to be found in the *combination* of the (objective) "Seven marks of behaviour", and consequently he did not distinguish between the notions of a primary and an ultimate goal as indicated above, nor between the types of quiescing 1 and 1a. Clearly, his notion of goal is *not* a teleological concept but what is now usually called a cybernetical concept, although formulated partly in mentalist terminology.

Much the same applies to Tolman whose system of theoretical concepts of instinct is, in fact, largely a strictly objectivist re-writing of McDougall's mixture of objectivist and subjectivist formulations. (Even the name of his doctrine "Purposive Behaviorism" he borrowed from McDougall.) Tolman did, however, recognise the distinction between the two behavioural notions of "goal" as discussed above,

and the corresponding distinction between two notions of "appetitive behaviour". In his account: "The ultimate to-be-got-to goal is, not the mere consummatory stimulus-object per se, but rather the final state of physiological quiescence to be reached by commerce with the consummatory object" (loc. cit., p. 272), which statement implies that what is called by TOLMAN the "consummatory response" is essentially identical with what here has been called a TAA type of *appetitive* behaviour, according to Type 1a of quiescing. For example: The ultimate quiescence of copulation is not the act but the physiological state of equilibrium of glands and sex organs, p. 277; the ultimate quiescence of sucking or eating is alimentary satiation, pp. 29–31 etc. (Cf. the concept of "extrinsic reward" in recent American learning theories.) Moreover, in order to verify his thesis, TOLMAN proposed as early as 1932 (p. 31) to undertake the decisive experiment carried out by JANOWITZ and GROSSMAN (1949) which largely proved his views, at least with regard to feeding behaviour, and with certain restrictions. For the rest, however, TOLMAN too was not fully consistent and did recognise the QSA type in amorous "contact-hungers" and in the "rest-hunger" (i.e. sleeping). Curiously, the parental appetite is missing from his enumeration without any argument or comment!

In the conceptual system devised by LORENZ and his adherents, on the other hand, although this was also derived from CRAIG, no type of quiescing was recognised, at least in theory, other than the QA type (Type II), i.e. a type which it is doubtful whether CRAIG himself ever actually discerned! "The goal is always the attainment of the stimulus situation required for the release of an instinctive action." "In man, the purpose of food acquisition is the instinctive performances of chewing, salivation, swallowing etc." "A grebe hunts for hunting's sake and does not perform these reactions more intensively when it is hungry." (Translated from Lorenz, 1937a, p. 298, 299 and 316.) "Every dog owner knows that being hungry or not exerts no influence whatever upon a dog's appetite for the instinctive actions of hunting." (Translated from LORENZ, 1937b, p. 31.) "The purpose of what CRAIG termed appetitive behaviour is the discharge of instinctive action." (LORENZ, 1950, p. 228.) "Strictly speaking, it is not the litter or the food the animal is striving towards, but the performance itself of the maternal activities or eating." (TINBERGEN 1951, p. 106.) "Such an end-response consumes the specific impulses responsible for its activation." (Loc. cit., p. 105.) "There is, in my opinion, no 'social instinct' in our sense. There are no special activities to be called 'social'." (Loc. cit., p. 112.) "There is no instinct for the selection of the environment." (Loc. cit., p. 113.) Obviously these and similar statements offer no scope for any type of quiescing other than Type II.

(Cf. the "intrinsic reward" in recent American learning theories.) In ethological practice, however, Type III (the QSA type) was also recognised, e.g. with regard to incubation in which the presence and number of eggs partly determines the amount of quiescing, as many bird breeders know. (See also BAERENDS 1955, 1956.)

Admittedly, in recent years some ethologists have started tentatively to recognize Type I (the QS type) of quiescing, at least in some instances of behaviour (BASTOCK et al. 1953, HINDE 1954, MOYNIHAN et al. 1954, THORPE 1954), but this recognition continues to be hesitating in several respects. For example, PRECHTL (1956), in an extensive review of neurophysiological aspects of ethology, does explicitly recognize both the QSA and the QS types of quiescing, but the instances mentioned by him (viz. coughing, sneezing, the bending reflex, fleeing, micturition, sleeping, feeding and incubating; pp. 292–293) all concern relatively short-term series of activities, mostly of a relatively simple type. No mention is made by him of the feedbacks originated by owning a nest, being paired, social gathering, social ranking, etc.; i.e. the "higher" and long-term types of QS endings. And further, the full bearing of the QS principle was quite recently again disputed by HINDE (1957) on grounds which for the most part consist in a confusion between the feedback principle as conceived by the present writer and the functional significance of behaviour[1]. It is in particular this confusion between the concepts of "feedback" and "function" which caused, and still causes, ethologists to hesitate to accept the QS type of quiescing, for usually the QS differs not so very much from what is biologically required.

It should be added here that, after reading this paper in manuscript, LORENZ, in a personal discussion with the writer, partly disagreed with the account of his ideas as given above. He pointed out that he certainly did recognise the existence of the "aversions" described by CRAIG and the "Appetenzen nach Ruhezustände" (i.e. appetites for quiescing situations) described by HOLZAPFEL, without, however, having published any research on them; and further he pointed out that the "optimum

[1] As mentioned already, HINDE's objections are also partly due to a misinterpretation of the present writer's views. And further, they are partly due to an undervaluation of the behavioural evidence available. For example, HINDE states (p. 117): "The cessation (in the female) of territorial defence when the young fledge is KORTLANDT's sole evidence that the rearing of chicks causes the ending of nesting behaviour." HINDE then suggests an alternative explanation by referring to a possible change in endocrine states. Actually, my original formulation was (p. 175): "After the young have fledged or prematurely left their nest by climbing or walking, territorial defence on the part of the female will cease", which statement clearly excludes a change in endocrine states since the female's parental behaviour is continued quite normally outside the nest, irrespective of the age at which the young leave the nest. Moreover, there is still other evidence. (See also KORTLANDT 1955, p. 176, and 1949, Chapters VIII and IX.)

taxes" distinguished by him (but rejected by TINBERGEN, 1951, p. 113) with regard to habitat selection should be regarded as identical with HOLZAPFEL's appetites for quiescing situations. By his statements on feeding behaviour as quoted above he had intended only to say that alimentary satiation will not prevent a predatory species from performing hunting behaviour, and he did *not* imply that a hungry predator would *not* hunt more intensively than a satiated one, nor did he imply that no feedback stimuli were involved in the termination of behaviour: his intention had been only to lay stress on an aspect which had been overlooked by nearly all previous authors on animal behaviour. For the rest he pointed out that his concepts should not be regarded as a closed conceptual system, and that in this respect many readers had misunderstood the tenor of his statements on these issues.

One can only regret that LORENZ never explicitly stated this point of view in a published paper, for by so doing he would have avoided much confusion and controversy, both among his pupils and among others. For example, HOLZAPFEL's considerations on the appetites for quiescing situations make clear that she thought her findings to be a critical revision and extension of LORENZ' concepts, instead of being an elaboration of his concept of taxis: "This particular kind of appetitive action and appetitive satifaction has until now not been demonstrated in this context" (i.e. in the context of LORENZ' concept of appetitive behaviour. Translated from HOLZAPFEL, 1940, p. 273.) Moreover, if we have to assume that LORENZ did recognise the appetites for quiescing stimuli under the heading of taxes, then we can only conclude that TINBERGEN misinterpreted both him and HOLZAPFEL when he wrote (1951, p. 106): "Lorenz has pointed out that the end of purposive behaviour is *not* the attainment of an object or a situation itself, *but* the performance of the consummatory action. HOLZAPFEL has shown that there is one apparent exception to this rule: appetitive behaviour may also lead to rest and sleep. This exception is only apparent, because rest and sleep are true consummatory *actions*." (my italics—A.K.). TINBERGEN's misinterpretation of the real tenor of HOLZAPFEL's argument is found in the fact that her term "Ruhe" means not so much "rest" but rather "quiescing" in general, as will be clear on careful study of her paper. And furthermore, if the concept of "consummatory stimulus" ought to fall under LORENZ's heading of "taxis", then it would also seem that BASTOCK et al. (1953, pp. 73–74), THORPE (1954, p. 103) and HINDE (1954, pp. 48–49) were all mistaken when they stated their arguments pointing to the existence of consummatory stimuli to be some sort of critical revision of, or addition to what THORPE called "LORENZ' thoroughgoing theoretical system of behaviour, more comprehensive than any which had preceded it." Evidently the views of ethologists concerning this item are not yet crystallised.

Anyhow, from all this we may conclude that, in ethological theory, generally speaking, (at least until recent years) CRAIG's idea stating that the appetitive behaviour may result *directly* in a consummatory end-stimulus according to what has been here called Type I of quiescing, was in the past either rejected, or neglected, or at least narrowed down so far as to apply only to those simple types of appetitive behaviour which involve no more than at most a choice between locomotory alternatives, such as, e.g., in habitat selection. And further, in the ethological concepts, the quiescing agency in food-seeking was usually assumed to be found in eating and not in alimentary homeostasis; the quiescing agency in nesting was postulated to consist in building instead

of in owning a good nest; the quiescing agency in fleeing was attributed to the act of fleeing instead of in being free from the predator; the quiescing agency of fighting was sought in discharging aggressive impulses instead of in being unthwarted or in having established a social organisation; and so on, often in plain contradiction of CRAIG's original formulations.

So we find that McDOUGALL and TOLMAN on the one hand, and the ethologists on the other hand, went opposite ways in the elaboration of their theoretical concept formation concerning the driving and quiescing agencies. Both groups branched off from CRAIG's comprehensive (although vague) outline, but, whereas the first focussed almost entirely on Type I and rejected Type II, the other concentrated mainly on Type II while neglecting Type I.

Naturally the divergences outlined above have important implications for the further conceptual extension of the theoretical systems concerned. Let us first consider McDOUGALL's system.

Since the "motor mechanisms" (in McDOUGALL's terminology, i.e. the AA and TAA actions in the above formulas) are driven by previously accumulated nervous energy springing from the E ("gas" in his model), but since the satisfying stimulus, when eventually attained, does *not* liberate *nor* consume the then remaining supply of energy, the question may be raised: What becomes of this energy? McDOUGALL makes clear that it may flow to other instincts, or flow back to a general source of energy, by means of a communication system between different reservoirs. HALDANE, in the paper quoted earlier, equated the phenomenon postulated by McDOUGALL with displacement phenomena, but actually it is nearly the opposite: The notion of displacement (at least as it was originally conceived) implies that *liberated*, *action-specific* impulses are thought of as *suddenly* sparking over, whereas in McDOUGALL's model *non*-liberated, *non*-specific energy is supposed *gradually* to flow from one reservoir to another. For further particulars the reader may be referred to Chapters II and IIIa of my paper on displacement (1940 b) in which I dealt at length with this distinction. (The difference runs parallel to the distinction between "impulse" and "tendency" which I made recently (1955).)

I am aware, of course, that my personal contribution to the notion of displacement was greatly inspired by McDOUGALL's idea of flowing energies, for I have personally always found McDOUGALL's conceptual system, generally speaking, more stimulating to theoretical penetration than the ethological system. Moreover, I am afraid that I am myself responsible for creating the confusion which I am now criticising; for apparently it was I who (on pp. 508 and 513 in 1940 b) introduced the pneumatic and hydraulic type of models on nervous energies which eventually superseded the chemical models used in ethology before that time. All these circumstances do not, however, alter the fact that the flowing energies are entirely different things in the two conceptual systems.

For the rest, it is a curious feature that the notion of displacement was explicitly suggested by CRAIG ("When the bird fails to obtain the appeted stimulus, the appetite sometimes disappears, due to fatigue or to drainage into other channels", loc. cit., p. 102.) but this idea was not taken over either by McDOUGALL nor by LORENZ, perhaps because it did not fit in too well with either McDOUGALL's QS type of

satisfaction or LORENZ' then assumption of reaction-specific stimulus *substances*.

A second important implication of the fact that McDOUGALL exclusively recognised the QS and QSA types of satisfaction is that in his concept genuine vacuum-activities cannot occur, for genuine vacuum performance implies not only the notion of "overflow" and discharge of an instinct-specific surplus of dammed up energy (which idea is perfectly compatible with McDOUGALL's system of concepts), but at the same time it also implies that this overflow and discharge *do quiesce* the appetite involved, and this latter characteristic is peculiar exclusively to the QA type which was rejected by McDOUGALL. His rejection of the quintessence of the notion of "Leerlaufen" constitutes indeed the real meaning of McDOUGALL's often misinterpreted statement: "The beast of prey hunts only when he is hungry." (Loc. cit. p. 101. A similar statement was made by TOLMAN, on p. 274.) For *if* it is (by definition) assumed that *only* an appetite for a QS or QSA is able to arouse the accessory appetitive and TAA behaviour peculiar to this appetite, then it necessarily follows that *seemingly* appetitive and TAA behaviour performed in the absence of the appetite normally arousing it must originate from *another* source and cannot be considered to be the appetitive and TAA behaviour which it seems to be. Consequently, what *we* would now call "vacuum hunting" was explained by McDOUGALL as "play" derived from some sort of general drive or over-all source of energy, according to the SPENCER-GROOS theory of play. That is to say, McDOUGALL did *not* deny the *facts* which we currently denote by the term "vacuum activities", but he explained them in a different way. In his own words (abbreviated): "The pursuer is not really hunting him; does not attempt to seize him and devour him. Play is a purposeless activity, striving toward no goal. Whence, then, comes the energy that sustains it? The answer is, I think, that the well-fed and well-rested animal, especially the young animal, has a surplus of nervous energy. It is the primal libido or vital energy flowing not in the channels of instinct, but overflowing, generating a vague appetite for movement and finding outlet in any or all of the motor mechanisms in turn". (Loc. cit. pp. 171–172.) Similar ideas were put forward by TOLMAN (pp. 278–279), and recently they have been revived by MEYER-HOLZAPFEL (1956). It will be clear how entirely different these ideas are in comparison with the ethological ideas on cumulation, flow and evacuation of nervous energies. They also differ from the type of flow from one reservoir to another as postulated by McDOUGALL and mentioned earlier.

Let us now consider some implications resulting from LORENZ's recognition of only the QA type of quiescence. Firstly, an important feature is that his conceptual system (at least as it was formulated by him in print) obviously offers no scope for assuming hierarchically superordinate steering or feedback mechanisms. For, *if* it is taken for granted that there exist no means of quiescence other than by discharging an action-specific amount of energy by means of merely running down this activity, "independently of releasing stimuli and independently of the total situation" (LORENZ 1937 b, p. 27), it naturally follows that all activities of the fixed pattern type are equal in rank and no superordinate goal-steering mechanisms can be conceived at all. In his own words (translated): "Superordinate instincts (as postulated by McDOUGALL) cannot be demonstrated. We would be justified in postulating them only if we could find a factor which exerts on the mosaic reactions a co-ordinating, purposive (zielsetzende) and wholistic (ganzmachende) effect. Such a factor can, however, never be found." (Loc. cit., p. 30.) It is true that in 1952 LORENZ did recognise the type of hierarchical organisation of "instincts" described by BAERENDS (1941) and TINBERGEN (1951), but *that* type of hierarchy is exclusively a hierarchy of *driving* (i.e. energy producing) mechanisms, and it cannot be considered to be a hierarchy of co-ordinating, purposive, wholistic or goal-steering (i.e. driving-*and*-feedback) mechanisms. In other words, the "choice" between different fixed patterns

of the AQ type is by the ethologists to this day necessarily assumed to be steered, *not* by any superordinate homeostatic or environmental need, but *only* by the RS's and E's of the fixed patterns involved. We will return to this point later.

A second implication of LORENZ' concept concerns the driving mechanism of appetitive behaviour. Since the appetitive behaviour is (by definition) supposed to be non-quiescing and, further, since quiescing is supposed to be identical to the discharge and consumption of nervous energy by the consummatory act, it follows that the AA in Type II is neither driven by, nor consumes energy from E. (The principle is in a certain sense the opposite of McDOUGALL's assumption according to which the AA does discharge and consume energy, whereas the QS does not; see the formula of Type I.) This raises the question: Whence does the appetitive behaviour in the ethological scheme obtain the energy driving it? LORENZ himself has never answered this question (see also LORENZ & TINBERGEN, 1938, pp. 23–26), but a solution was offered by TINBERGEN in his centre theory (1951, Fig. 97): The energy is supplied by *another* E, namely a superordinate centre in a hierarchical driving organisation. Consequently TINBERGEN's scheme requires *two* levels of centres in a *one*-level behaviour organisation, *three* levels of centres in a *two*-level behaviour organisation and so on. (See his Fig. 98.) The idea is somewhat reminiscent of McDOUGALL's explanation of "play" by postulating an over-all source of general energy, for in both authors those facts which do not fit into their too rigid schemes are explained by postulating an ad hoc extra source of energy. A formula may be set out as follows:

$$\begin{tikzcd}
 & & & o & \\
(PS) \arrow[r] & E_{\text{extra}} \arrow[r] \arrow[dr] \arrow[ddr] & E \arrow[r] & RS \arrow[r] & \mathbf{QA} \\
 & & AA_1 \arrow[ur] & & \\
 & & AA_2 \arrow[uur] & &
\end{tikzcd}$$

This formula does not, however, solve the whole problem: it is true that it does explain why the AA can still be performed in certain cases when the E → RS → QA part of the system is exhausted, but it does not sufficiently explain why, in many other cases, the intensity with which the AA is performed appears to be closely correlated with the amount of action-specific energy accumulated in the E → RS → QA part of the system.

MAJOR AND MINOR INSTINCTS

This brief review may suffice to demonstrate how entirely different are the concepts of instinct as elaborated by McDOUGALL and LORENZ, notwithstanding their common origin and notwithstanding the apparent resemblances suggested by certain homonymous verbal formulations. Wherever one looks at these conceptual systems, it is always differences that one comes across. And further, McDOUGALL's instincts are *major* instincts, superordinate steering mechanisms activating or inhibiting the fixed patterns and other mechanisms normally subservient to them; and, as a direct result of these postulated cybernetical mechanisms in their relation to the environment, the major instincts will guide the animal towards the fulfilment of *those* physiological and ecological requirements for which such mechanisms are

equipped, such as, for example (at least in *some* species), filling the stomach, genital contact, owning a nest, social gathering, social ranking, and so on. LORENZ' concept, on the other hand, deals with *minor* instincts, independent and uncontrolled single patterns, each working on its own account and for its own sake, without being themselves controlled by any superordinate feedback mechanism as to their ultimate homeostatic or environmental result with regard to filling the stomach, genital contact, owning a nest, social gathering, social ranking, and similar physiological and ecological requirements. Parenthetically, neither of these authors attempted definitely to prove or to investigate whether "his" type of instinct was the *only* one in existence.

Furthermore, this review may also demonstrate how much LORENZ has deviated from the ideas conceived by CRAIG, from which all the concepts discussed here were derived. For, according to CRAIG's account, such fixed patterns as, for example, "display" and "billing" in doves are assumed to be appetitive behaviour in the service of the consummatory action of copulation, i.e. they are assumed to be subservient to major instincts, instead of being single and independent "mosaic" patterns. So it is not only with regard to the postulated quiescing agencies but also with regard to the postulated integrational level of behaviour that LORENZ has diverged from CRAIG. Some passages in CRAIG (pp. 98 and 103) might even be considered as suggestive of a hierarchy of goals *and* feedback mechanisms, i.e. a type of hierarchy which certainly does not fit in with LORENZ' conceptual system and which has always been firmly rejected by all orthodox ethologists.

At this point (if not much earlier) the reader might object: But, considering that the ethological conceptual system has now become fairly well established, what is the point of digging up and airing, at great length, such old and obsolete concepts and controversies? Well, I myself do not feel that they are yet so very obsolete, nor do I think that the ethological concepts are yet so very firmly established. Few people, even among professional workers, seem to be aware of the real situation in this respect, and I had an interesting experience on this point. In 1955, at the Third Ethological Conference at Groningen, an esteemed British colleague said to me: "Is it right what I have heard, that you recognised the consummatory stimulus as long ago as 1940?" He simply did not know that the notion had been current in Great Britain for at least thirty or forty years, and that it was exported from there to Holland and elsewhere. It seems inconceivable that this knowledge should have apparently sunk into oblivion in Britain, considering that METHUEN & Co. have sold 187,615 copies of

McDougall's books to date (12 titles[1]), and this takes no count of the numbers sold by other publishers (8 titles, estimated at more than 80,000 copies at the least).

TWO SCHOOLS, AND AN ATTEMPT AT SYNTHESIS

In Holland in the late thirties, when I was a young amateur student of cormorant behaviour, there was quite a lot of scientific discussion on the items considered above. On the one hand there was a school of workers centred round the Amsterdam Zoo and led by Bierens de Haan and Portielje, who were especially alive to the evidence pointing to "purposiveness", or "purposivity", or "directiveness" or "directedness" (or whatever other word one may prefer) in animal behaviour. (German: Zielstrebigkeit, Zielgesteuertheit. Not to be confused with such notions as teleology, functional adaptation, survival value, Zweckmässigkeit, Zweckdienlichkeit, etc.) In other words, these workers were especially aware of the *major steering processes* recognisable in animal behaviour. Consequently, according to their view, the different units of behaviour (i.e. the instincts) should be defined by the goals at which and by which these units were supposed to be steered. Since, however, at that time, cybernetics did not yet exist, goal-seeking and learning machines could hardly be envisaged. For this reason it is no wonder that these senior workers became adherents of a vitalism of the mentalist stamp as represented by McDougall, implying that purposiveness and learning could be accomplished only by non-physical, psychic or mental agencies. They subscribed to McDougall's key argument on this item: "Purpose implies foresight". Parenthetically, there were other reasons too: Anybody who has had a good deal of experience with the larger Zoo animals will know that, when dealing with such animals, he is obliged to be a mentalist if he wishes to preserve life and limb! (See also Bierens de Haan, 1935.) The formula of behaviour adopted by this school comes down to the types i, ia and iii, i.e. the QS and QSA types, applied to major instincts. (It is true, Bierens de Haan was not quite consistent in following McDougall's criteria, for on pp. 82–85 of his book on instinct (1940) he caused confusion by mixing up the QS type of quiescing with a teleological classification.)

On the other hand, at that time in Holland there was, at the University of Leiden, a group of workers led by N. Tinbergen and stimulated by Lorenz in Austria, who were less aware of purposiveness, or who were at any rate less concerned by the then non-existence of cyber-

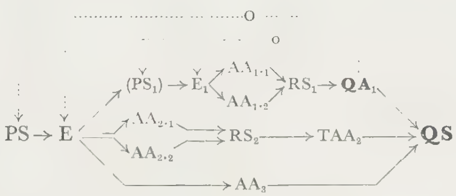
[1] The writer expresses his thanks to Messrs. Methuen & Co. for giving this information.

netics, and who consequently felt justified in accepting as a working hypothesis ZIEGLER's (1910) chain-reflex theory, i.e. the PS → R → QS formula, together with the objectivist and mechanist doctrines. These workers were interested not so much in the goals, i.e. the "final causes" or feedbacks which steer and terminate a given train of behaviour, but rather in the "primal causes", i.e. the factors which start and activate a given train of behaviour, the releasing mechanisms and internal drives etc. Eventually, after 1934, the notion of purposive behaviour as conceived by CRAIG and called "appetitive" behaviour, gradually penetrated into the concepts outlined by this group, and in 1937 (b) LORENZ (on the authority of VON HOLST) rejected ZIEGLER's theory; but all the same, purposive behaviour was accorded only the place of a long-term program-objective of research as being the "non-analysed residue" (unanalysierter Restbestand, loc. cit., p. 39) instead of being made the quintessence of the concept of instinct; and further, no purposive behaviour was recognised other than that resulting from minor instincts of the fixed pattern type, according to formula II, together with some purposive behaviour resulting from minor subordinate or interlaced taxis components which are variable in spatial direction but not in pattern.

The Amsterdam school of workers liked to call themselves "animal psychologists". Affiliated to this group, but holding somewhat different theoretical views, were BUYTENDIJK and VERWEY. The Leiden group preferred to call themselves "ethologists", and sometimes they claimed their branch to be identical with the scientific study of behaviour. Between the two groups there was much exchange of argument, especially on the metaphysical problem, but also on behavioural items such as the question whether the "purposive" (or "appetitive") behaviour is directed to, and ended by, major QS or minor QA goals. The two groups held that the terms "animal psychology" and "ethology" indicated entirely different doctrines, and consequently they were inclined to overlook the existence of such "objectivist animal psychologists" and "purposive behaviourists" as, for example, CRAIG and TOLMAN, and many other American and European colleagues who did not fit into the simplified two-school dichotomy.

When I was confronted with this controversy at that time, neither of the parties could offer conclusive arguments as to the *metaphysical* question how to explain purposiveness in behaviour. Moreover at that time such things as self-guiding missiles and learning robots were mere fantasies in the science fiction of the day. One could at most choose a working hypothesis. All the same, my cormorants offered what seemed to me very decisive evidence on the *behavioural* question of whether purposiveness did really exist. Their behaviour clearly appeared to be

organised in large purposive (or "appetitive") systems, in such a way that the behaviour belonging to a system was evidently steered by, among other things, integrative cybernetical mechanisms (or "psychisms") of the major QS and QSA type as recognised by McDougall. The *degree* of integration differed, however, from one case to another, and from this it became obvious that, within the major systems, there also existed certain minor purposive (or "appetitive") systems which, according to the circumstances in the case under consideration, were able to pursue their own goals on their own account. These minor systems corresponded to the QA type as described by Lorenz. Consequently I came to recognise what I then called "concentric appetites" or "concentric purposive systems" (1940 a). Nowadays one might call them hierarchical systems of driving *and* feedback mechanisms. In these systems several quiescing agencies of different types (I, Ia, II and III) may be organised in different, more or less complicated, ways. Some basic combinations of behaviour units are rendered in the following formula. (The scheme may be extended by adding to it more units either in parallel or in series.)



Some examples may be elucidatory here. Major quiescing agencies of the QS type are, in the cormorant: absence of predators, presence of social companions, having a full stomach, owning a nest, being paired, etc. A major quiescing agency of the QSA type is cloacal contact in copulation. (Pair-formation and copulatory behaviour are, in the cormorant, separate major systems, but they share many minor systems.) In the nesting system, a minor quiescing agency of the QS type is fasten*ed* twigs in the nest, a minor TAA act is fasten*ing* twigs, a minor QA act is quivering twigs, and a minor QSA act is bringing twigs to the nest. That is to say, every twig of the nest must stick fast, and normally the quivering is a movement specifically conducive to fastening, but there is no special appetite for performing the fastening (inserting) movement as such, although there is a special appetite for performing the quivering movement. Thus a cormorant when in a specific mood for quivering may vacuum-quiver with twigs that are already sticking fast, but when *not* in a quivering mood, he may leave to his mate the fastening of the twigs he brought in his bringing mood, and for the rest the fastening movement as such shows no threshold fluctuations, whereas the quivering movement as such does show threshold fluctuations.

Some other QA acts which participate in major QS systems are, for example: the precipitous side-slip (in fleeing behaviour), hunting for fish (in feeding behaviour) and, to a lesser degree, "gargling" (in pair-formation). All these activities are characterised by the fact that under constant environmental and homeostatic circumstances they will show threshold-fluctuations and vacuum-performances, even

if their normal goal, the major QS, is constantly present. (E.g. zoo cormorants fed by hand ad libitum will from time to time perform false-hunting in a pond without fish, and paired cormorants will from time to time "gargle".) If, however, their major QS is absent, then these activities will be performed very much more frequently and intensively, or even practically inexhaustibly, and it is in this latter respect that these activities differ essentially from the LORENZ type of QA activities which are not arranged in a QS system.

Some TAA activities are: flying rapidly away, horizontally (from danger on the ground), and, at least in young birds, begging for and swallowing fish when being fed by the parents. These activities are as stereotyped as all fixed patterns are, but the characteristics of central nervous action-specific threshold fluctuations, exhaustibility and vacuum-performance as found in QA activities are lacking in them. For example, a young cormorant when not fed will go on begging indefinitely, when continually stuffed it will never beg, and when forced to regurgitate after feeding, it will at once beg and swallow food again. In this respect, begging young cormorants behave quite differently from young passerines, in which the "gaping" seems to be little or not at all motivated by hunger. (At least according to LORENZ, 1950, p. 259; see however PRECHTL, 1953.) Another stereotyped AA activity which is, however, not a TAA act, is "wing-flapping": as long as there is no female, the wing-flapping will go on indefinitely (alternated with gargling), and as long as a female is present it will never be shown.

And, finally, some minor QSA activities are: gnawing fish, incubating and, presumably, mounting when considered as a partial pattern of copulation. These activities cannot be performed at all without an appropriate object or, if otherwise, when being performed "in vacuo", they appear not to quiesce the state of unrest (e.g. in false-incubating).

The decision on which of the above types a given activity belongs to is, at least in theory, quite easy: one has simply to find out which combinations of factors are able to start *and* stop the sequence or series of behaviour in which this particular activity is normally framed, and what consequences follow if the goal is attained or missed, etc. And similarly, according to the same principles, the stimuli are divided into PS, RS and QS stimuli, and S components in QSA acts. In practice one is, however, often unable to separate *all* the initiating and feedback factors involved, and consequently some of the examples mentioned above are still somewhat provisionally classified because of incomplete evidence. And further, there are several activities with regard to which even a tentative classification is as yet impossible (e.g. feeding the chicks). All the same, the distinction of these types in themselves may be considered to be sufficiently established.

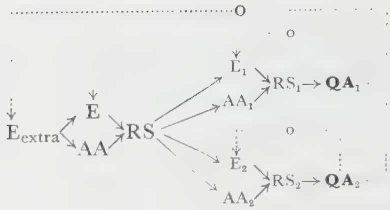
To me personally the conceptual system of flowing energies and quiescing agencies as summarised above, and here illustrated and elaborated by means of a formula, seemed at that time a fairly acceptable one. Obviously there was really nothing new or revolutionary in this system: it was simply a combination of the pre-existing ideas as outlined by CRAIG, McDOUGALL and LORENZ. This combined concept was intended only to account for the full factual evidence advanced by these senior authors and their adherents, and demonstrated as well by my cormorants. Certainly I should never have been either interested in or able to draw up this scheme if I had not been greatly encouraged at that time by many stimulating personal discussions, in particular

with the holders of such very different views, Dr. PORTIELJE and Dr. N. TINBERGEN, who opened my eyes to the merits, inadequacies and inconsistencies in the two pre-existing concepts. At any rate, the existence of major purposive systems according to the AA → QS type of quiescence was so conspicuous in other animal species as well, as has been argued by many other writers of widely different origin, that it seemed difficult to imagine that it could ever be overlooked.

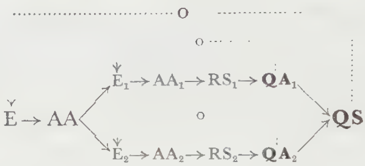
CONTINUATION OF THE CONTROVERSY ON THE QUIESCING AGENCIES, OR GOALS OF BEHAVIOUR

Curiously enough, the inner circle of ethologists continued for many years to disregard the evidence pointing to the existence of the major AA → QS systems. I have often wondered why, and it is still a mystery to me. The behavioural facts were indeed clear enough, they were expounded often enough, and they were discussed often enough, from different sides, both publicly and privately. For example, M. HOLZAPFEL (who cannot be regarded as an orthodox ethologist) argued cogently in favour of the existence of the AA → QS type of quiescence in sleeping, fleeing, pair-formation, territorial and infantile behaviour (1940). Since then some ethologists stated sleeping to be an action—while at the same time the whole of the remainder of HOLZAPFEL's argument continued to be unnoticed, at least until recent years. (See, e.g., TINBERGEN, 1951, p. 106). And similarly the evidence advanced by American Tolmanians also remained unnoticed. Since then the consummatory stimuli have been restored to honour, at least to some extent, by some ethological authors, without actually going into the contributions of previous authors on this theme.

It is true on several points there has come a rapprochement of views. In Holland, on the side of the "animal psychologists", the existence of the LORENZ type of QA instincts has become recognised. The "ethologists", on the other hand, recognised the existence of major instincts in a hierarchy of driving E mechanisms of the following type. The formula renders a *two*-level behaviour organisation according to TINBERGEN's model. (For printing reasons the formula is simplified by not splitting up the AA into AA_1 and AA_2, the AA_1 into $AA_{1,1}$ and $AA_{1,2}$, etc. as was done in the preceding formulas. Further, the PS, PS_1 and PS_2 components are omitted.) It may be added that in the original formulation by BAERENDS the RS (i.e. TINBERGEN's "blocks", see his Fig. 97, 1951) did not exist at the higher and intermediate levels, so that the AA guided the animal directly to the PS_1 or the PS_2.



For easy comparison, a similarly simplified two-level AA → QS system as conceived by the "animal psychologists" and containing, apart from that, the *same* behaviour units may be rendered as follows:



It will be clear that this latter scheme allows for a hierarchical organisation of feedback mechanisms governed by homeostatic and/or environmental needs and controlling the QA patterns, which type of hierarchy is lacking in the ethological scheme. Moreover, whereas in the ethological concept the superordinate instinct may be said to be *less* specialised than the subordinate ones, in the latter concept the superordinate instinct is conceived to be *more* specialised than its subordinates because it is thought to be controlled by a *more* complicated and *more* rigid system of releasing and feedback stimuli than its subordinates. (See also Kortlandt, 1955, pp. 210–212.)

The main controversy, however, goes still further: According to the evidence put forward by the "animal psychologists" there often occurs yet a *third* way of quiescing the E, either naturally or experimentally, namely by means of an AA_3, which should be inserted into the last formula and which leads *directly* from the E to the QS, as indicated in the last formula but two. According to the "ethologists", on the other hand, at least until recently, neither this AA_3 nor the QS existed at all. In their concept no provision was made for an ultimate feedback mechanism controlling the final homeostatic or environmental *result* brought about by the animal's behaviour. For example, food-seeking was supposed to occur "absolutely independently of the state of the animal's hunger" (Lorenz 1937 a, p. 316), and "strictly speaking, it is not the food the animal is striving towards, but the performance itself of eating." (Tinbergen, 1951, p. 106).

Another difference comes to light if for the moment the ramifications

in the two hierarchy formulas are left out of consideration. In the ethological scheme the distinction between two different levels then disappears, the E and the E_1 coincide or at least become indistinguishable, nothing is left of hierarchy, and the intensity with which the QA is performed is determined exclusively by the intensity of the E (or E_1) and the RS (or RS_1) according to LORENZ' "principle of dual quantification" (1950, p. 251 ff.). In the second behaviour formula, on the contrary, if the ramification is left out of consideration, we retain a *non-ramified two-level hierarchy* (to be briefly called the AA → QA → QS type) in which the intensity with which the QA is performed depends on *three* factors, viz. the E_1, the RS_1 (i.e. the RS), and the absence of the QS (in the E → QS system), so that a *triple* quantification is required. For example, the intensity of hunting (or false-hunting) in cormorants depends on the stimulus value of hunting objects, the specific hunting energy (or hunting appetite) *and* the superordinate state of food-hunger (or food-appetite). Similarly, the intensity with which the cormorant's nest-building is performed (taking all building activities together) is determined by the stimulus value of the twigs, the strength of the internal action-specific building drives, *and* the absence or incompleteness of the nest. And similarly, the intensity of courting depends, in the cormorant, on the "charms" of the partner, the damming up of the drive since the last flirtation *and* the condition of being non-paired. (Quite apart from still other factors, such as the strength of the individual temperament.) It will be clear that this principle may have important implications, not only for research problems, but also for practical applications, e.g. with regard to the problem of the sex drive in bulls used for artificial insemination. (Parenthetically, in bulls there is yet a fourth factor, probably located in the IRM, as can be inferred from a remarkable paper by ALMQUIST and HALE, 1956. One might perhaps even go so far as to doubt, on grounds of the evidence produced by these authors, whether the motor act of copulation in bulls can, strictly speaking, be considered to be a consummatory act at all. On the contrary, it seems rather that the decrease of responsiveness following copulation in bulls is entirely due to some kind of nervous exhaustion or adaptation in the IRM, and to sperm depletion. If so, this would imply that the motor act of copulation in bulls is a pure reflex-type of TAA act.)

At first glance one might here object that in a non-ramified hierarchy the distinction made above between the presence of an RS_1 and the absence of a QS is superfluous, for one might argue that (in certain instincts) the RS_1 and the QS appear to be the counterpart of one another, and if so the said distinction would in reality be a tautology; or one might argue that (in certain other instincts) the RS_1 and the

absence of the QS can be conceived to be two stimuli working on an equal footing, and if so the principle of heterogenous summation would suffice. Such objections would not, however, be sound. Firstly, in cases when the quiescing end-stimulus is attained *directly* by means of the appetitive behaviour (i.e. via the AA_3 route in the last formula but two) the $E_1 \rightarrow RS_1 \rightarrow AQ_1$ part of the system is short-circuited, but nevertheless the degree of quiescing attained appears to be fairly complete. (E.g. when a cormorant in the Zoo is fed by hand, or when a wild cormorant robs a nest.) Thus it follows that the absence of the QS acts *indirectly* (via the E) upon the $E_1 \rightarrow RS_1$ link, whereas the RS_1 is of course supposed to be directly linked with the E_1. (A live fish *necessarily* releases hunting, a twig *necessarily* releases building, etc., when the bird is in the proper mood.) Secondly, such objections would not account for the difference in the motivational mechanisms involved. A Zoo cormorant fed by hand to satiation does occasionally hunt, thereby proving that the E_1 is really distinct from the E and that we are therefore justified in speaking of a non-ramified two-level hierarchy. Similar instances are found in nesting and pair-forming behaviour. (E.g. nest-owners that bring twigs, that perform vacuum-quivering, etc.) Thirdly, and most important of all, such objections would not account for the difference in selectivity at the different levels. A cormorant in a non-hungry hunting mood (when fed by hand in the Zoo) hunts for twigs, straw, string and similar oblong objects, whereas a cormorant in a hungry hunting mood will never do so, however hungry it may be. Evidently the appetite for food is much more selective than the appetite for hunting in itself, and so here again we are justified in postulating a two-level non-ramified hierarchy of integrative agents provided with distinct feedback mechanisms. Similar phenomena are found in nesting, pair-forming, copulatory and parental behaviour. When surveying them one finds that as a rule the superordinate mechanisms are more selective than their subordinates, although in certain respects the reverse is found in a few instances.

Admittedly this matter is rather complicated. At any rate, to my knowledge, *no other* attempt has ever been made to try and give a consistent theoretical formulation of the behavioural data dealt with here. For the rest, the reader may be referred to the complete data to be published in future papers.

For the sake of completeness, and in order to settle certain existing misunderstandings, a small digression may be inserted here. The type of hierarchy with which the ethologists have particularly been dealing may be called a hierarchy of driving-*non*-feedback mechanisms. The type of hierarchy which I described in 1940 and which was rendered in the last formula may be called a hierarchy of driving-*and*-feedback mechanisms, or a hierarchy of goals. In the latter type the "choice" between different behavioural alternatives, as well as the starting and stopping of all these alternatives

taken together, is assumed to be determined *not only* by the feedback mechanisms which are peculiar to each separate behaviour mechanism in itself, but *also* by a superordinate feedback mechanism which reacts upon the ultimate homeostatic and/or environmental situation, either achieved by all these behaviour alternatives taken together or resulting from other circumstances. Consequently, the superordinate feedback mechanism is assumed to be controlled by *more* (and more complicated) causal factors as compared to the subordinate ones, and in this respect the latter type of hierarchy is exactly the *opposite* of the former one.

There does occur, however, yet a *third* type of hierarchy, namely a hierarchy of *motor integrational levels*, which in several respects resembles the second type but in which the superordinate mechanism *does not choose* between the partial patterns subordinate to it. During ontogeny, and also in seasonal maturation in birds and fishes, one often observes that one partial pattern (either AA_1, TAA_1 or QA_1) and another partial pattern (AA_2, TAA_2 or QA_2) fuse together into one single and undivided whole pattern, either $AA_{\Sigma(1+2)}$, $TAA_{\Sigma(1+2)}$ or $QA_{\Sigma(1+2)}$. This phenomenon may be called the principle of isolated development followed by ascending integration. During seasonal "dismaturation", and also in cases of ill-health or "psychotrauma" e.g. sudden loss of nest or young, the total pattern will again partly or largely break up into its composing partial patterns. Correlated with these fusion and fission processes, certain other integrative and disintegrative phenomena are observed. These integrative and disintegrative processes have been extensively studied in man by psychoanalysts and clinical psychiatrists. In the cormorant I considered them particularly in a Dutch paper (1949) which was summarised on pp. 189–196 of an English paper (1955) where other references are also mentioned. (Among others, BINGHAM, EIBL-EIBESFELDT and SAUER.) Such an experimental neurological approach to this same type of hierarchy of integrative mechanisms was developed in particular by W. R. HESS (1943), building on previous workers.

At first glance the Σ type of integrative phenomena may seem to be suggestive of the following behaviour formula:

$$\begin{tikzcd}[column sep=small]
 & & E_1 \arrow[r] & AA_1 \arrow[r] & RS_1 \arrow[r] & \mathbf{QA}_1 \arrow[dr] & \\
E \arrow[r] & AA \arrow[ur] \arrow[dr] & & & & & \mathbf{QA}_{\Sigma(1+2)} \\
 & & E_2 \arrow[r] & AA_2 \arrow[r] & RS_2 \arrow[r] & \mathbf{QA}_2 \arrow[ur] &
\end{tikzcd}$$

On second thoughts, however, this formula will appear to be misleading since, in *this* type of behaviour integration, the QA_1 and QA_2 *disappear* when the $QA_{\Sigma(1+2)}$ emerges in the course of ontogeny and maturation, and the reverse is found in dismaturation and trauma. Consequently, the QA_1 and QA_2 do not stand in a strict means-end relation to the $QA_{\Sigma(1+2)}$, and therefore *this* type of hierarchy (although it *is* a sort of hierarchy) cannot be regarded as a hierarchy of driving-and-feedback mechanisms, at least in the present state of research. I regret that in previous papers on hierarchy I did not sufficiently sharply distinguish between the type of hierarchy in which the $AA_1 \rightarrow QA_1$, $AA_2 \rightarrow QA_2$ etc. are *chosen* and *controlled* by a feedback mechanism of the QS type while remaining *distinct* patterns, as opposed to the type of hierarchy in which the $AA_1 \rightarrow QA_1$, $AA_2 \rightarrow QA_2$ etc. *disappear* owing to their *amalgamating* into the $AA_\Sigma \rightarrow QA_\Sigma$ system. The difficulty, however, is that (in the course of ontogeny, maturation and dismaturation) the last two types (or perhaps all three?) develop and regress according to the *same* principles of isolated develop-

ment followed by ascending integration, and conversely. Consequently, in observational practice, one is often unable to draw any sharp line between the last two types. Anyhow, from what might be called a purely "clinical" point of view, the difference between the types of hierarchy (2) and (3) is less wide than the formulas and theoretical considerations here offered may suggest. And perhaps they are less different in their fundamental nature too. More experimental research will be needed on this item.

CAUSES OF THE BIAS AGAINST GOAL-STEERED BEHAVIOUR

After this digression we may now return to our earlier topic, namely the puzzling question why so many students of behaviour have continued to disregard, for so many years, the evidence available on the AA → QS type of quiescence as expounded by the Craig-McDougall-Tolman school. One can hardly imagine this to be due to the verbal confusion about the meaning of the term "consummatory". Nor does it seem plausible to suppose that the animal species studied by ethologists happened to provide less evidence of the AA → QS type than the species studied by other authors. Such statements as, for example, asserting that hungry predators do not hunt more eagerly than satiated ones are simply not true, except maybe in domestic animals. One can only wonder how such statements could ever be made.

Perhaps an explanation may be found in Lorenz' recent admission (1955, p. 176): "I was biased by my grudge-fight against vitalism." It is true that on that occasion Lorenz was referring to his adherence to the chain-reflex theory in the early thirties. Such attitudes may, however, often survive their initial grounds and origin.

How much Lorenz has continued to be biased against purposiveness can easily be demonstrated from his indictment read at the Fourth Symposium of the Society for Experimental Biology (published 1950).

Firstly, according to his account (pp. 227–228 and 262–263) the vitalist "purposive psychologists" have confused the survival value of behaviour with the end or goal aimed at by the animal itself. In his own words: "In the opinion of purposive psychologists, the 'infallible' instinct is telling the organism what it has to do. Therefore it is only consistent to identify the survival value with the end or goal at which the organism as a subject is aiming" (p. 228). "These conceptions have been arrived at from finalistically considering what the animal *ought* to do in order to sustain itself and its species", instead of "from what it actually *does*" (p. 262). It is difficult to understand how Lorenz could arrive at such an idea, for it is hardly possible to imagine a more unwarrantable criticism. However, Lorenz has expressed it so often and so emphatically, on different occasions, that many of his adherents have come to believe in the correctness of this critique. (See, e.g., Tinbergen, 1951, p. 3–4; Baerends, 1956, p. 14.) Lorenz does not quote exactly, by publication and page, which purposive psychologist mentioned in his bibliography has ever made any statement even suggesting what is asserted by Lorenz. Personally I have never

come across such a statement, with the sole exception of pp. 82–85 in Bierens de Haan (1940) who there defends, from a theoretical point of view, a teleological classification of instincts, but rejects it from a practical point of view. (At any rate, this passage in Bierens de Haan is clearly inconsistent with the general purport of his book.) And for the rest, all these purposive psychologists have always strongly argued that an instinct is defined, objectively, by the quiescing stimulus or act, and, subjectively, by the satisfying stimulus or act "which will bring the train of activity to a close" and "which alone can allay the appetite" (McDougall, 1923, pp. 110 and 119). Evidently these criteria refer to what nowadays would be called steering mechanisms, and they have in themselves nothing to do with survival values. If otherwise, the purposive psychologists would *not* have distinguished, for example, the sexual and the parental instinct, or the fighting and the dominance instinct.

It seems rather to have been Lorenz and his adherents who, when reading McDougall's writings, confused the notion of purposiveness with survival value. And McDougall himself appears to have foreseen the possibility of this confusion, so to speak in anticipation of Lorenz' critique, as may be illustrated by the following passages: "Some of the mechanists would scornfully repudiate the claim that we may reasonably regard animal behaviour as purposive. If they admit the word 'purposive', they define it in a non-psychological manner, and describe as purposive all the actions which seem to be serviceable to the life of the animal or the species". (Loc. cit., p. 49.) [Parenthetically, such a misleading definition of "purposive" is actually found in Tinbergen, 1951 (p. 3–4), but elsewhere (on p. 105–106) Tinbergen used this term in the same meaning as McDougall did.] After having made the quoted statement, McDougall went on further to argue that his (objectivist) "seven marks of behaviour" cannot be explained by mechanist principles, "at least in the present state of science." At the end of the same chapter he added: "A word must be said here on the meaning of the word 'teleological'. I have insisted that all mental activity is purposive, that it is a striving toward a goal, however vaguely the goal may be thought of. The word 'teleological', which means directed toward a goal, has often been applied to animal behaviour, or to the realm of life in general, to imply that the processes of organisms are adjusted to bring about certain results which are the goal designed or willed by the Creator. We may, however, use the word 'teleological' as equivalent to 'purposive' in the sense in which it is defined in this chapter. The difference between the two meanings is very important". Etc. (Loc. cit., p. 71.) Shortly afterwards, evidently in order to prevent confusion arising from the twofold meaning of the term "teleological", McDougall proposed to call his doctrine "the hormic theory".

Secondly, in his same critical review quoted above, Lorenz complains of a lack of knowledge of animal behaviour among the purposive psychologists (and among other workers) in the following passage (p. 233): "It was a really crushing blow to cherished ideals when, as a young student, I first realised that the great authorities on 'instinct', such as Lloyd Morgan and W. McDougall, *did not know* the relevant facts about innate behaviour with which I, ignorant boy though I was, was mentally struggling even then; reactions not attaining their goal because of lack of intensity, vacuum activities and the innumerable ways in which innate behaviour patterns were miscarrying, were evidently unknown to the great theorists."

Now we have already seen that McDougall in a certain sense *did* recognise vacuum activities but explained them as "play" according to the Spencer-Groos theory, which fact was apparently overlooked by Lorenz. But apart from this, even if I can partly share Lorenz' feelings on this point, it would seem to me that this way of evaluating one's contribution to the knowledge of animal behaviour is not quite just. Behaviour is so immensely complicated that we should perhaps judge

our colleagues only by what they *saw*, and *not* by what they overlooked. And thus we have to acknowledge that the vitalistic animal psychologists did see the major integrative and purposive (i.e. *goal-steered*, hence not teleological) systems, perhaps because their metaphysical beliefs made them liable to see these things. For the rest, a good theorist is not necessarily a good observer, and vice versa.

A third objection raised by LORENZ against the non-ethological schools asserts that these workers failed to treat the behaviour of a species as an organic system. According to his own words: "If there is one behaviourist or reflexologist, or one purposive psychologist, who began by observing all there is to be observed in a species, making an inventory of its system of actions before forming a working hypothesis, then I am very sincere in asking his forgiveness; I am not a very well-read man and have failed to hear of his existence. But all other professional students of animal behaviour have been guilty of the one unpardonable offence against the most fundamental law of inductive natural science: they have one and all formed a hypothesis *first* and proceeded to look for examples to confirm it *afterwards*." (Loc. cit., p. 233, see also pp. 262–263.)

In reality, I am quite sure that LORENZ personally knows at least some "purposive psychologists" who did thoroughly know their animals, and did strictly apply this very same broad approach as advocated by LORENZ; and some of these workers are indeed included in the bibliography accompanying LORENZ' paper. We can therefore only conclude that LORENZ, in the heat of his crusade against epistemological and metaphysical aspects, fell a victim to some kind of unconscious "scotomization" regarding the real contributions to the knowledge of animal behaviour, as well as with regard to the factual amount of evidence on purposiveness, produced by the purposive psychologists. (*Postscript:* After having read this paper in MS, LORENZ pointed out to me that, in the critisized passage, he did *not* allude to certain continental European workers to whom this passage might seem to refer but whom he does *not* consider to be "professional" students of animal behaviour, in the strict sense. Moreover, he drew my attention to the fact that he recently (1957) evaluated McDOUGALL's merits. While taking note of this explanation, my impression still is that LORENZ at that time, in consequence of his bias against vitalism, underrated the validity of purposive psychology as a conceptual system.)

For the rest, we may fully agree with the exclamation which LORENZ made in his charge against teleology: "We have particularly urgent reasons to want insight into the causal context of 'instinct'. With atomic bombs in its hands and with the endogenous aggressive drives of an irascible ape in its central nervous system, modern humanity is a whole or system which has got very thoroughly out of balance. No teleological meditation will help us, we shall have to *do* something about it, and this implies the necessity of causal insight." (Loc. cit., p. 229.) We may even go further and add to LORENZ' statement a hint on the way in which something can perhaps be done: Firstly, there do exist at least some mammalian species (e.g. domestic cattle) in which the endogenous aggressive QA drives are controlled and checked by superordinate QS drives directed towards social stability to such an extent that, even in cases of a change in the "positions of strength", renewed fighting and social re-ranking seldom occurs, and only after much time has elapsed. (G. C. JANSEN and R. J. VAN BIEZEN) In such species the goal of fighting is not (or hardly) fighting in itself but rather establishing once and for all a social organisation which makes fighting superfluous. And secondly, there do exist quite a lot of human culture patterns (especially among the most primitive peoples!) in which no signs of overt endogenous pugnacity seem to be detectable at all. (See, e.g., VAN DER BIJ, 1929.) Thus we may be pretty sure that in man too there does exist a mechanism which, under certain social conditions, is fully able to check the QA type of fighting

behaviour. Or perhaps we may equally argue the other way round and conclude that, at least in man, the so-called endogenous aggressiveness is not so much a product of instinct but rather a product of certain unknown social conditions resulting from civilisation and disintegrating the checks of instinct.

CONCLUSION

Even after this digression demonstrating that the "grudge-fight" against vitalism and purposivism is still being fought by some ethologists, the reader may nevertheless still object: But are not these out-of-date discussions and controversies on doctrines and concepts, as brought up again by HALDANE and the present writer, really obsolete nowadays?

They are certainly not. The term "appetitive behaviour" was re-introduced by the ethologists in the thirties, by way of some sort of verbal magic, in order to escape from the vitalist and mentalist associations often involved in the then current synonyms "purposive" or "directive" or "goal-seeking" behaviour. Nevertheless, right up to today, appetitive behaviour has continued to bear a hereditary taint and it has never become a favourite in ethology. In 1937 b LORENZ dedicated a chapter to "The Duty to Analyse" appetitive behaviour, but in 1951 TINBERGEN wrote that appetitive behaviour "baffles the scientist" (p. 106). Thus little real progress seems to have been made on this point in the interim. Appetitive behaviour has indeed continued to be the CINDERELLA of ethology and a corpus alienum in its conceptual system. One even searches in vain for clear criteria to *define* the notion of appetitive behaviour in ethology. (Variability? Inexhaustibility? Adaptability? Directiveness? That which leads to a quiescing mechanism? Or a combination of these criteria? Compare McDOUGALL's clear-cut "Seven marks of behaviour.") The Cambridge round table conference on nomenclature (1949) arrived at the following definition of appetitive behaviour: "The essential content of the term when used in connection with instinct is that it is a variable means to an invariable end. There was fairly general agreement that the term might be defined as follows: The variable introductory phase of an instinctive behaviour pattern or sequence." (THORPE, 1951, p. 37.) Clearly, these are two *contradictory* paraphrases, for "a variable means to" is a much narrower idea than "a variable introductory phase", whereas "an invariable end" (by which a QS ending is *in*cluded) is a much wider idea than "an instinctive behaviour pattern or sequence" (by which a QS ending is *ex*cluded). And further, how is one to classify the TAA act in a TAA → QS or TAA → QSA sequence? According to the Cambridge definitions, such a TAA act would seem to be *neither* appetitive behaviour nor a consummatory act. Is it then a reflex? But according to the Cambridge definition, a reflex "specif-

ically involves a part only of the organism", which statement obviously does not apply to the TAA act. In recent years, HINDE (1953) and VAN IERSEL (1953) found the distinction between appetitive behaviour and consummatory act to be a difference of degree rather than absolute; and this finding (though correct in itself) was of course more apt to conceal than to emphasize the difference between the two notions. Subsequently, BAERENDS et al. (1955, p. 328) attempted to redefine the appetitive behaviour as "behaviour in which the S.A.P. increasing effect supersedes the S.A.P. reducing action", but such a definition in terms of the motivational effect (thought apt to account for the feedback principle, and clearly including the TAA act) nevertheless leaves out what originally was the core of the notion of appetitive behaviour, namely the combination of variability with purposiveness.

In short, owing to fear of metaphysical implications, the whole notion of appetitive or purposive (i.e. goal-steered) behaviour, together with the problem of the quiescing agencies and the integrative systems, is still completely in the air in ethology. Worse still, on *these* items, modern ethology is still behind the point reached by purposive psychology, as may be illustrated by considering that TOLMAN's essential distinction between the primary and the ultimate goal of appetitive behaviour has not even entered into ethology today. A thorough re-evaluation of all the existing vague concepts on all these points is imperatively needed. Such a re-evaluation will indeed necessitate a straightforward tackling of the whole problem of *purposiveness*. The ominous word will have to be frankly uttered.

Nobody need be frightened about this. The situation is very different now from that of the twenties and thirties. Again, such things as goal-steered missiles and learning automata do exist nowadays. So, in the untroubled atmosphere of pure science, we may feel safe for that at least. Cybernetics has given us a promising approach for tackling purposiveness. Goals can now be conceived of as *causes*, without having recourse to metaphysics. Consequently, objective research on purposiveness now has, in principle, nothing more to do with the *metaphysical* doctrine of vitalism, nor with the *methodological* doctrine of mentalism. Nor is there any longer any reason for "grudge-fights" against these and similar theoretical doctrines. The ghosts have vanished at last. Nowadays we are allowed to take positive evidence for what it is.

Or rather, the ghosts have not all quite vanished yet. If we are talking about automata with a computor engineer, he will quite unconcernedly tell us that his machines "observe", "ascertain", "reject", "remember", "learn", "gamble", "communicate", "are instructed", "aim at", "try and err", "have a programme", "search in their memory", "control themselves", "compare their result with

the set task", and so on. If we then ask the engineer if he is using these terms only as crude analogies to mental phenomena, he will say: "No. Electronic brains are doing exactly the same as human brains. They have been designed and instructed to do just that." If we then further ask whether his machines experience mental phenomena too, he will reply: "I don't understand what you mean by that. That is not my concern." In the field of animal psychology, however, we have not yet advanced so far. A prominent American colleague once said to me: "I would not dare to use such mentalistic terms. If I did I should be kicked out of my laboratory!"

Of course we need not be romantic about those classical authors who devoted their lives to building up a thorough-going conceptual system of purposiveness, such as, for example, the CRAIG-McDOUGALL-TOLMAN trio. Nor need we go back as far as ARISTOTLE or SAMKHYA philosophy. We should do better to be critical and eclectic, taking available evidence for what it is, and wherever it can be found, in both classical and recent authors, in order that we may all see the concrete facts pointing to the existence of the major purposive and integrative systems so conspicuously operating in animal behaviour, but so often still overlooked. To quote CHRISTOPHER SMART (1722–1771): "Nature is more various than observations, though observers be innumerable."

SUMMARY

This paper aims at reviewing and clarifying some ambiguous and controversial notions which, in the past, have given rise to persistent confusion. Special attention is given to the notion of "appetite" or "instinct-specific energy"; the notion of "purposive" or "appetitive" or "goal-steered" behaviour; the notion of the "goal" or "end" or "purpose" of this behaviour; and the notion of "consummation" or "satisfaction" or "quiescing."

Much misunderstanding has been caused because homonymous or similar technical terms may, in different conceptual systems, often have quite different technical meanings. Therefore the basic driving and feedback mechanisms as postulated in the concepts of instinct devised by CRAIG, McDOUGALL, TOLMAN, LORENZ and later authors are expounded by introducing a system of behaviour formulas. The implications hidden in these concepts are investigated and the inconsistencies involved in them are elucidated. Since this review is itself a summary, it is inappropriate to summarise it further here.

As a result of these considerations it appears that the main difference between the conceptual systems considered is found in the type of behavioural evidence which was mutually overlooked in them. An

attempt at synthesis aimed at accounting for the full evidence available leads to the statement of a principle of triple quantification replacing LORENZ' principle of dual quantification. Three types of hierarchy of instincts are distinguished and discussed in this connection. With regard to TOLMAN's distinction between the primary and ultimate goal striven after by the organism, two different types of goal-steered (i.e. appetitive or purposive) behaviour involving different types of feedback mechanisms are distinguished, and, further, the notion of the "terminal appetitive act" is introduced. Other current concepts are re-evaluated. In future research more consideration should be given to the definition and elaboration of the concept of goal-steered behaviour.

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FEEDING HABITS AND ZONATION IN SOME INTERTIDAL SNAILS

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I. INTRODUCTION

It is a well-known fact that the algae growing in the tidal zone show zonation. This zonation is, of course, due to a passive selection of diaspores by the environment. The abiotic factors constituting part of the environment of each zone are largely connected with the tidal rhythm, which causes a different degree of immersion, emersion, exposure to sun, rain and wind, etc., at different heights of the tidal zone. As the properties of the different species of algae are different as regards tolerance towards the abiotic factors, the algae are caused to grow in vertically restricted zones. Besides, a certain degree of competition between the algae may play a role in the origin of the zonation.

An equally well-known fact is the zonation of a number of intertidal

snails. Doubtless, abiotic factors operate in a similar way on the vertical distribution of the snails, but certain habits of the different species of snails (like geotaxis or phototaxis) as well as competition also may influence their distributions over the different zones. In some cases a correlation may exist between the zonation of the algae and the zonation of the snails.

If such a correlation exists, it may be asked whether the snails are dependent on the algae on which they live, and when this question can be affirmed, the problem arises on what this dependence is based. The animals may depend on the seaweeds for a number of reasons, e.g. for food or substratum. In this case a certain preference may exist for the seaweeds on which the animals live, feed and/or spawn.

This investigation is only concerned with the problem to what extent certain habits of the snails (e.g. geotaxis, phototaxis, feeding preferences etc.) operate to limit the natural habitats of the species concerned. Tolerance experiments have not been made. In the case of a feeding preference we may ask if the distribution of the seaweed-feeding species is correlated with a certain preference for the algae on which they are found in nature, and if these algal belts are bordered by algae which the snails do not like. Together with abiotic factors, such a difference in attractiveness between the algae might be very important in limiting the natural habitats of the snails concerned. Deliberately emigrating or dislodged snails will not settle in algal zones beyond the preferred ones when they have the opportunity to perceive and reach their favoured food plants and when their preference for these plants is strong.

II. ACKNOWLEDGEMENTS

This paper contains observations and experiments, which were made during a two months' visit to the Station Biologique at Roscoff in June and July, 1957.

Sincere thanks are due to the Roscoff committee of the Royal Dutch Academy of Sciences that enabled me to undertake this investigation, to the Dutch Ministry of Education, Arts and Sciences for granting me a subsidy, to the scientific and technical staff of the Station Biologique, especially to Prof. P. Drach, whose advice and critical remarks were very helpful, to Dr. C. O. van Regteren Altena, who has checked my identifications, to Prof. Dr. D. J. Kuenen and Dr. J. Verwey for their valuable criticism of the manuscript, and last but not least to my wife, who has assisted me enthousiastically with the somewhat tedious task of sampling and counting.

III. VERTICAL DISTRIBUTION OF INTERTIDAL SNAILS

A. METHODS

In order to obtain an insight into the distribution of intertidal snails over the common algal belts, a number of samples were taken in three transects on Ile Verte. This island lies just opposite the Station Biologique, protected against heavy surf on all sides. Similar samples were taken on the coast of the Bay of Douarnenez near Argol in SW Brittany.

Sampling was done in a rather primitive way: in each algal zone all snails were collected that could be found during 15 minutes searching by two persons. As the *Patella* species are not easily dislodged, these species are left out of the picture. Afterwards the snails of each sample were identified and counted.

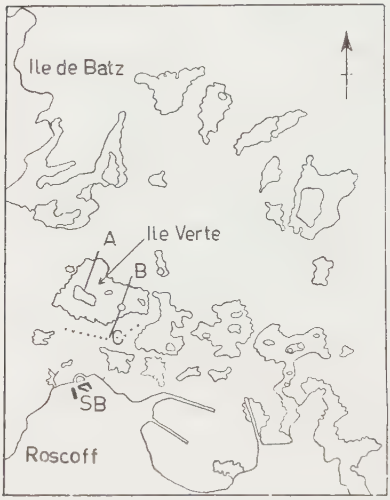

Fig. 1. Ile Verte. A, B and C: transects studied. ...: Creek with *Zostera*. SB: Station Biologique.

Some disadvantages of this method should be mentioned here:

1. The area searched is not the same in each algal zone, as searching takes more time among large plants (e.g. *Fucus vesiculosus*) than among small ones (e.g. *Chondrus crispus*). The figures obtained do not indicate real population densities expressed as a certain number of animals per unit of surface.

2. Size and colour of the animals have probably great influence on their chance of being found. Care should be taken not to overlook small species or juvenile specimens when sampling is done in a zone where adult specimens of large species are very abundant.

3. Numbers collected within the fixed sampling time of 15 minutes are also influenced by the habits of the snails. When they mount the plants when the tide is out, and can thus be found on top of the weeds, fully exposed to the daylight, the numbers which can be picked up are extremely large. This is the case in *G. pennanti* for instance. The temptation to collect the greatest possible number of these snails within the available time of 15 minutes must be strongly resisted, to make it possible to undertake a complete and thorough inspection of the whole plant and the substratum, where other species spend their more secluded life.

4. It is difficult to estimate psychological factors which may play a role in this way of sampling. Enthousiasm or fatigue may influence the intensity of searching to a large extent.

In spite of these objections the sampling method described has been chosen on account of the following considerations:

1. It is almost impossible to use the well-known sampling method of collecting all animals that occur within a frame, enclosing a fixed surface area, even when this frame is made rectangular in stead of square, allowing for the limited width of some of the algal zones. This may at first seem rather surprising, but a moment's reflection will make this clear. The very structure of the rocky shore on which the zonation to be examined can be found, makes the use of frames rather difficult, because all kinds of irregularities, such as boulders, clefts, tidal pools, steep walls, etc., interrupt the algal belts almost everywhere.

2. Another sampling method is the determination of the number of animals per unit weight of algae. This method may be applied to the study of the microfauna, like COLMAN (1940) has done. For the study of the macrofauna it is, however, completely inadequate, because it is too laborious when large quantities have to be taken. Moreover, when the samples are taken, the snails will fall off and cannot be retrieved quantitatively.

The method used gives no more than the qualitative distribution of the different species in the intertidal zone, and some approximation of the abundance of each species in the different belts.

TABLE I

Transect A, Ile Verte: N of Western part

decreasing altitude →	very irregular steep rocks						gentle slope with sandy bottom and boulders				
snails / zone	*Pelvetia canaliculata*	*P. canaliculata* and *F. spiralis*		*Fucus spiralis*	*A. nodosum* and *Fucus vesiculosus*		*Fucus serratus*	*Rhodymenia palmata*	*Bifurcaria tuberculata*	*Chondrus crispus*	*Laminaria digitata*
		P. can.	*F. spir.*		*A. nod.*	*F. ves.*					
ittorina rudis	378	33	2	—	—	—	—	—	—	—	—
Ionodonta lineata	—	9	7	6	—	—	-	—	—	—	—
ittorina nigrolineata	—	37	18	7	—	—	—	—	—	—	—
ittorina obtusata	—	—	55	104	61	189	5	2	—	—	—
ibbula umbilicalis	—	—	43	96	46	44	13	—	—	—	—
ucella lapillus	—	—	1	1	3	3	—	—	—	—	—
ibbula pennanti	—	—	—	—	—	2	309	262	430	386	399
ibbula cineraria	—	—	—	—	—	—	7	35	14	11	146
alliostoma zizyphinum	—	—	—	—	—	—	—	2	4	5	—
rivia arctica	—	—	—	—	—	—	—	—	—	2	—
hasianella pullus	—	—	—	—	—	—	—	5	—	—	-
atina pellucida	—	—	—	—	—	—	—	—	—	—	2

Figure I represents Ile Verte; the letters A, B and C refer to the transects investigated. The result of this sampling work can be found in tables I, II and III.

B. DESCRIPTION AND DISCUSSION

A survey of tables I–III shows us a very beautiful zonation of both seaweeds and snails.

1. *Pelvetia canaliculata* zone

The zone of *P. canaliculata* is the highest one. This alga has to sustain long periods of emersion and can dry up so much as to become crispy. Subsequent immersion apparently causes total recovery. In the lower part of this zone a certain degree of mixing occurs with plants of the next zone: that of *Fucus spiralis*. In transect A the upper part of the *P. canaliculata* zone has been studied separately. It is inhabited only by *Littorina rudis*[1] as is clear from table I. This species does not feed

TABLE II

Transect B, Ile Verte: NE of central rock

decreasing altitude →	rather steep rock				gentle slope with sandy bottom and boulders							
snails / zone	*Pelvetia canaliculata*	*Fucus spiralis*	*A. nodosum* and *F. vesicul.*		*F. vesiculosus* and *F. serratus*		*Fucus serratus*	*F. serratus* and *Enterom.*		*Rhodymenia palmata*	*B. tuberculat* &*Himanthali*	
			A. nod.	*F. ves.*	*F. ves.*	*F. ser.*		*F. ser.*	*Ent.*		*B. tub.*	*H. lor*
Littorina rudis	140	8	—	—	—	—	—	—	—	—	—	—
Monodonta lineata	9	15	1	—	—	—	—	—	—	—	—	—
Littorina nigrolineata	11	24	3	—	—	—	—	—	—	—	—	—
Littorina obtusata	—	115	76	140	81	73	9	2	—	1	—	—
Gibbula umbilicalis	—	11	11	15	16	35	8	11	—	—	—	—
Littorina littorea	—	—	—	—	—	2	1	—	—	—	—	—
Nucella lapillus	—	1	3	—	—	3	2	1	—	—	—	—
Gibbula pennanti	—	—	—	—	3	35	125	223	26	229	350	12
Gibbula cineraria	—	—	—	1	—	—	9	20	1	41	5	—
Calliostoma zizyphinum	—	—	—	—	—	—	1	2	1	3	4	—
Ocinebra aciculata	—	—	—	—	—	—	1	—	1	1	5	—
Trivia arctica	—	—	—	—	—	—	—	—	—	—	3	—

[1] Prof. P. DRACH allowed me to make use of a report by one of his pupils, CLAUDE DEYGLUN, called: "Biologie comparée de deux sous-espèces de *Littorina saxatilis* (Olivi)." This paper has not yet been published. The two subspecies in question, *L. rudis* and *L. nigrolineata*, are very different in quite a number of respects. For that reason specific rank is claimed for them. The major differences between the species are, in short:

L. rudis	*L. nigrolineata*
shell colour white	shell colour yellowish
no black spiral stripes	distinct black spiral stripes
viviparous	oviparous

on the seaweed and can be found in large numbers in crevices and even on bare rock exposed to sun and rain. It is not confined to the *P. canaliculata* zone, being abundant also somewhat higher on the shore beyond the algal belts. In the lower part of the *P. canaliculata* zone in transect A, and in the same zone investigated in transects B and C, two other species of snails appeared: *Monodonta lineata* and *Littorina nigrolineata*[1]. These two species, like *L. rudis*, are never found on the seaweed, so I do not think they feed on it.

2. *Fucus spiralis* zone

The narrow zone of *F. spiralis* follows immediately below the *P. canaliculata* zone. Here two other species of snails can be found: *Littorina obtusata* and *Gibbula umbilicalis*. The former feeds exclusively on the seaweeds and attains here rather large numbers. The figures in tables I–III clearly demonstrate that *L. obtusata* almost never occurs on *P. canaliculata*. *Gibbula umbilicalis* is often found on the rocky substratum. The numbers of *L. rudis* have dropped enormously.

3. *Ascophyllum nodosum – Fucus vesiculosus* zone

Descending further we reach the next zone, where we find a vegetation of two large algae: *Ascophyllum nodosum* and *Fucus vesiculosus*. In all three transects they were mixed up to a large extent, but some difference may be noted: *A. nodosum* generally grows more on top of big boulders, lying amidst a vegetation of *F. vesiculosus*.

But for the casual occurrence of the carnivorous dogwhelk, *Nucella lapillus*, this zone does not provide novelties. However, an interesting difference can be seen in the numbers of *L. obtusata* on the two plants, its density being always higher on *F. vesiculosus*.

Species from higher zones disappear almost completely (*L. rudis*, *M. lineata* and *L. nigrolineata*).

4. *Fucus vesiculosus* zone

As *F. vesiculosus* at Ile Verte descends much lower into the tidal zone than *A. nodosum*, we can find an almost pure vegetation of *F. vesiculosus* beneath the former zone. It has been investigated at two different heights in transect C (table III). The blunt periwinkle, *L. obtusata*, is very abundant at the higher level and devours the seaweed to such an extent that sometimes only the midrib, the airbladders, and the reproductive organs are left. The number of *L. obtusata* is much lower at the lower level. Here also another *Gibbula* can be found, i.e., *G. pennanti*. This species predominates in numbers throughout all lower zones.

[1] cf. note on preceding page.

TABLE III

Transect C, Ile Verte: SW of central rock

decreasing altitude →	rather steep rocks				
snails / zone	*Pelvetia canaliculata*	*Fucus spiralis*	*A. nodosum & F. vesiculosus*		*Fuc[illegible] vesicu[illegible]*
			A. nod.	*F. ves.*	
Littorina rudis	38	8	—	1	—
Monodonta lineata	30	10	1	2	—
Littorina nigrolineata	7	28	2	1	4
Littorina obtusata	3	187	211	301	319
Gibbula umbilicalis	—	5	6	9	48
Littorina littorea	—	—	—	—	—
Nucella lapillus	—	—	—		3
Gibbula pennanti	—	—	—	—	—
Gibbula cineraria	—	—	—	—	—
Calliostoma zizyphinum	—	—	—	—	—
Ocinebra aciculata	—	—	—	—	—
Cantharidus striatus	—	—	—	—	—
Bittium reticulatum	—	—	—	—	—

5. *Fucus vesiculosus – Fucus serratus* zone

Below the almost pure vegetation of *F. vesiculosus* we find a zone in which *F. vesiculosus* and *F. serratus* are mixed. This zone has been investigated in transects B and C (tables II and III). The numbers of *L. obtusata* on the two algae do not differ very much; *G. umbilicalis* is in transect B more abundant on *F. serratus*, and in transect C on *F. vesiculosus*, whereas *G. pennanti* is in both cases more abundant on *F. serratus*.

6. *Fucus serratus* zone

The zone of *F. serratus* is most distinct in transects A and B (tables I and II). The numbers of *G. umbilicalis* and *L. obtusata* drop enormously here and *G. pennanti* becomes the dominant species. We find also some individuals of another *Gibbula: G. cineraria*, which species, however, attains its greatest density apparently lower.

The delicious looking bright green *Enteromorpha*, which grows between *F. serratus* (and *F. vesiculosus*) is not much frequented by *G. pennanti*, as is demonstrated in tables II and III.

7. *Rhodymenia palmata – Bifurcaria tuberculata – Himanthalia lorea – Chondrus cricpus* zone

Below the *F. serratus* zone we find in transects A and B a zone in which a number of seaweeds occur, appearing more or less in the sequence indicated in the above heading. On all these seaweeds *G. pennanti* is the most numerous species, but its numbers are very low on *H. lorea*.

gentle sandy slope with pebbles				shallow creek with sandy bottom (E.)			same creek, more W, deeper	
[F]ucus [vesi]culosus	*F. vesiculosus & F. serratus*		*Enteromorpha*	*Zostera marina*	*Ulva lactuca*	*Laminaria saccharina*	*Zostera marina*	*Laminaria saccharina*
	F. ves.	*F. ser.*						
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
78	74	78	—	—	—	—	—	—
67	147	85	—	—	—	—	—	—
1	—	4	—	—	—	—	—	—
4	4	2	—	—	—	—	—	—
21	85	149	41	600	59	76	65	203
—	—	—	—	1	8	6	67	89
—	1	1	—	—	—	—	—	—
—	—	—	—	—	1	—	2	—
—	—	—	—	—	—	—	64	2
—	—	—	—	~	—	—	—	~

Other snails that can be found here are *G. cineraria*, *Calliostoma zizyphinum*, *Trivia arctica*, *Ocinebra aciculata* and *Phasianella pullus*. Except the first, none of them attains large numbers.

8. *Laminaria digitata* zone

The lowest zone investigated in transect A is the *L. digitata* zone, which is only exposed at extreme low water of spring tides. Here, too, *G. pennanti* is the most numerous species, but the numbers of *G. cineraria* have also increased greatly. Most animals can be found on the fronds of the leathery and shiny seaweeds, especially at their withered tips. A novelty here is *Patina pellucida*, a species which is confined to this zone.

9. *Zostera marina* bed

At the south side of Ile Verte lies a shallow creek with a large bed of *Zostera marina*. This creek is, at its eastern part, almost completely uncovered at low tides, except at low water of neaps, whereas the western part remains largely covered, even at low water of spring tides. Samples were taken at two different places, one in the eastern and one in the western part of the creek. The difference between these places is apparent, as may be seen in table III. *Cantharidus striatus* is only found in the deeper part of the creek. This species is known for its strict dependence on *Zostera*. The numbers of *G. cineraria* are much higher in the deeper part.

In considering the numbers of *G. pennanti* it should be taken into account that individuals of this species mount the plants when the tide

is out and the plants become exposed to the air. Consequently, they can be seen in countless numbers on the *Zostera* leaves which lie flat on the sandy bottom of the eastern part of the creek. The number collected is thus always far higher than when the snails have to be sought in the immersed *Zostera* plants in the western part.

Between the eelgrass, but generally somewhat deeper, often in depressions made by the tidal current around boulders in the creek, a large number of other seaweeds occur. Only *Ulva lactuca* and *Laminaria saccharina* have been examined. *G. pennanti* and *G. cineraria* are particularly abundant at the withered tips of the fronds of *L. saccharina*. Unfortunately, the numbers of *Bittium reticulatum* have not been estimated, as this beautiful snail can not be sampled quantitatively, by the method used, on account of its small size.

Summarizing, we see in all three transects essentially the same distribution of the snails in the algal belts.

As has already been said, similar samples were taken on the coast of the Bay of Douarnenez near Argol in SW Brittany. Here, the vegetation is in some ways different from that which we encountered on Ile Verte, since this place is far more exposed to the influence of the waves.

Two inlets were examined; fig. 2 shows the situation. The abundance of barnacles, mussels and dogwhelks expresses clearly the exposure to the surf. In the eastern part of the second inlet *F. spiralis* and *P. canaliculata* are replaced by *Lichina pygmaea*. Table IV gives the numbers of snails in the samples from the algal belts examined.

The species of Gastropods are largely the same as those on Ile Verte. Only *L. rudis* and *L. nigrolineata* could not be found here. Their places in the tidal zone are occupied by another (sub-)species of the *L. saxatilis* group, whose shells are blackish, dark-green, brick-red, orange or whitish. I have not tried to find any correlation between their colour and zonation.

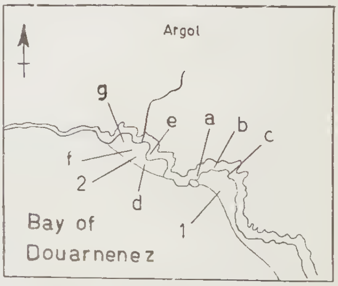

Fig. 2. Coast of the Bay of Douarnenez near Argol. 1 and 2: inlets. a–g: sampling. places. a: *F. serratus;* b: *F. serratus;* c: *F. serratus* and *F. vesiculosus;* d: *A. nodosum;* e: *L. pygmaea;* f. *F. spiralis;* g: *P. canaliculata.*

Another novelty is the small *Lacuna pallidula*, which occurs on *F. serratus* growing in the first inlet. This species here generally has a green shell, though some yellow ones may found too. At the inner end of this inlet *F. serratus* and *F. vesiculosus* grow together. The density of *F. obtusata* is much higher here on the latter.

Summarizing, the following list of the distribution of the different snail species in the algal belts can be given:

Littorina rudis	From the *F. spiralis* zone to far above the *P. canaliculata* zone. Not feeding on the large weeds. Very abundant.
Monodonta lineata	Almost confined to the *P. canaliculata* and *F. spiralis* zone. Not feeding on the large seaweeds. Abundant.
Littorina nigrolineata	In the lower part of the *P. canaliculata* zone and in the F. spiralis zone. Not feeding on the large seaweeds. Abundant.
Littorina obtusata	On *F. spiralis*, *F. vesiculosus*, *A. nodosum* and *F. serratus*. Largest numbers on the first two algae. Feeding on the seaweeds. Very abundant.
Gibbula umbilicalis	In the *F. spiralis*, *F. vesiculosus* & *A. nodosum*, and *F. serratus* zone. On rocks and on the algae. Abundant.
Littorina littorea	Found in small numbers in the zones between *P. canaliculata* and *F. serratus*. Not feeding on the large seaweeds.
Nucella lapillus	From the *F. spiralis* to the *F. serratus* zone. Carnivorous. Common.
Gibbula pennanti	On a large number of seaweeds below the upper part of the *F. vesiculosus* zone. Attains enormous numbers somewhat lower down. Avoids *H. lorea* and *Enteromorpha*. Apparently feeding on the large seaweeds, but perhaps only eating the microscopic epiphytes. Very abundant.
Gibbula cineraria	On a great number of seaweeds, from *F. serratus* and lower. Feeding on the large seaweeds, but maybe only consuming the microscopic epiphytes. Abundant.
Ocinebra aciculata	In the lower part of the intertidal zone often on boulders. Common.
Cantharidus striatus	Exclusively found on *Zostera marina* which is constantly immersed. Abundant.
Calliostoma zizyphinum	In the lower zones, below *F. serratus*. Common.
Bittium reticulatum	Abundant on *Zostera*, etc.
Trivia arctica	In the lower zones. Not very common.
Phasianella pullus	Below the *F. serratus* zone. Not very common.
Patina pellucida	On the fronds of *Laminaria digitata*
Lacuna pallidula	On *F. serratus*. Not found on Ile Verte. Herbivorous.

Of the numerous problems that arise from the observations reported in this chapter only the feeding preference of *L. obtusata*, in connection with its vertical distribution, and some ecological differences between *G. umbilicalis* and *G. pennanti* have been chosen for further investigation here.

C. APPENDIX: THE COLOUR VARIETIES OF LITTORINA OBTUSATA

The following discussion is concerned with the significance of the colour varieties of *L. obtusata*. Such a discussion has become necessary, as Barkman (1955) believes to have found proportional differences in

TABLE IV

Samples from a place on the coast of the Bay of Douarnenez near Argol

decreasing altitude →	inlet 1				inlet 2 Western part		inlet 2 Eastern part	
snails / zone	*F. vesiculosus & F. serratus*		*Fucus serratus*	*Fucus serratus*	*Pelvetia canaliculata*	*Fucus spiralis*	*Lichina pygmaea*	*Ascophyllum nodosum*
	F. ves.	*F. ser.*						
Littorina saxatilis	—	—	—	—	393	42	262	—
Monodonta lineata	—	—	—	—	19	23	112	—
Littorina obtusata	103	13	85	11	—	216	7	217
Gibbula umbilicalis	40	8	19	13	—	13	79	72
Littorina littorea	—	—	—	—	3	13	5	—
Nucella lapillus	1	—	3	39	—	—	—	2
Gibbula pennanti	1	11	86	17	—	—	—	1
Gibbula cineraria	—	1	—	—	—	—	—	—
Lacuna pallidula	—	3	18	11	—	—	—	—
Ocinebra erinacea	—	—	—	1	—	—	—	—

the vertical distribution of these colour varieties. Moreover, he has tried to demonstrate differences in food preference between var. *citrina* and var. *olivacea.*

Everybody who has had the opportunity of examining a large number of shells of *L. obtusata* will have been impressed by their striking variability in colour. DAUTZENBERG and FISCHER (1914) have distinguished 11 colour varieties within the species. Although some authors (e.g. VAN BENTHEM JUTTING, 1933) are of the opinion, that they have exaggerated, several of the varieties are generally recognized.

BARKMAN (1955), in his extensive work on *L. obtusata*, says (p. 25): "The possibility of phenotypical variation may be excluded. ...current velocity, salinity and illumination have no influence upon the colour of the shell in the genus *Littorina*. Other important habitat factors might be the food (i.e. the species of algae consumed) or the level in the tidal zone, on which depends a complex series of factors, such as desiccation, temperature, fresh water influence, and illumination. We can find all colour varieties, however, in conjunction with every species of alga and at every level".

Notwithstanding the last remark, he attaches much value to these varieties, saying that "part of the varieties differ not only in a morphological, but also in an ecological respect".

As regards his distinction between var. *citrina* and var. *olivacea*, however, I only partly agree with his argumentation. BARKMAN rejects the possibility that var. *citrina* represents a juvenile form of var. *olivacea*, when he writes:

"One might be inclined to believe that the variety *citrina* represents

a juvenile form of the variety *olivacea* in view of the following facts:
1. Specimens of *citrina* are generally smaller than those of *olivacea*.
2. Individuals of an intermediate colour, marbled yellow and green ones, and green animals with a yellow zone near the mouth, can all be found, whereas yellow specimens with a green growing zone do not occur.

Nevertheless, I am of opinion that *citrina* is a distinct, independent variety, since:
1. One will find big yellow specimens and small green ones.
2. The calcareous inner layer of the shell is white in *citrina*, dark violet in *olivacea*, which is visible on the parietal innerside of the mouth. It may also be seen on the outer surface of the shell after removal of the conchyoline layer with acid.
3. The animals of the variety *citrina* are unpigmented and cream coloured, those of *olivacea* are highly pigmented and dark grey or black. Transitional forms between the two extremes do indeed exist, but they are far less frequent than those between the extremes of shell colour.
4. In several regions the variety citrina is the only one found".

Primarily, it should be emphasized that a hard-and-fast delimitation of the colour varieties is not possible at present, owing to the extremely gradational characters. Colour and pattern may show almost every possible combination. Besides, the colour of the animal may vary largely independently of the shell colour. Perhaps a genetic research like that of STAIGER (1954) on *Nucella lapillus* would enable us to order the chaos.

TABLE V

Four samples of *L. obtusata* from different algal belts in transect C, divided roughly into the 3 major colour groups and into two size groups

	Fucus vesiculosus		*Fucus vesiculosus*		*Fucus vesiculosus* and *Fucus serratus*			
					F. ves.		*F. ser.*	
total height	>8 mm	<8 mm	>8 mm	<8 mm	>8 mm	<8 mm	>8 mm	<8 mm
Colour groups of *L. obtusata*								
Yellow or orange	90	41	27	110	2	57	3	48
Olive green	55	3	10	4	1	1	1	7
Brown or almost black	120	10	13	14	1	12	3	16

BARKMAN's arguments merit some remarks:
1. It is probable that the juvenile individuals of var. *olivacea* are yellow and not distinguishable from the young *citrina* specimens. This is demonstrated in table V, where the numbers of *L. obtusata* found in

4 samples in transect C have been roughly divided into the 3 major colour groups and into 2 size groups. It is very clear that:

a. comparatively, the proportion of small (juvenile) specimens[1] is highest in the lowest zones,
b. the number of yellow juvenile specimens is 4 times greater than those of the olive green and brown ones together, and
c. in the higher zones the proportion of larger yellow specimens is not higher than that of the olive green or brown ones.

As in the higher zones the number of medium-sized or large olive green specimens is rather high, it is surprising that we do not find a reasonable number of olive green juveniles somewhere in the tidal zone. The few that have been found are out of all proportions to the number of larger ones. The assumption, that most of the very small specimens of var. *olivacea* are yellow, may be a reasonable explanation.

It agrees with BARKMAN's remark that specimens of var. *citrina* (yellow) are generally smaller than those of var. *olivacea* (olive green) and with his observation that var. *citrina* is most abundant on *F. serratus*. This alga occupies the lowest belt in which *L. obtusata* occurs and here we find always a large number of juvenile (yellow!) specimens.

The unequal distribution of animals of different ages over the tidal zone in *L. obtusata* is very apparent. It will be demonstrated in *G. umbilicalis* too (Chapter v). It looks as if the animals gradually migrate upwards in the tidal zone when growing older.

2. BARKMAN remarks that animals of var. *olivacea* are highly pigmented and dark grey or black. This may be true for the surroundings of Den Helder, where BARKMAN did the main part of his work, but it is not so in Roscoff, where most olive green shells contain cream-coloured animals, as in var. *citrina*. The animals with a very dark shell (dark brown or almost black) are always black.

In the preferendum experiments to be discussed below I used animals collected on a single species of alga. These animals naturally belonged to all different colour varieties, but, as may be seen from the results, this fact has not seriously influenced the experiments. This neglect of colour varieties may be considered as a step backwards in comparison with BARKMAN's work, but the extreme difficulties to be encountered in distinguishing exactly these varieties left no other way out. Moreover, the differences in food preferences between var. *citrina* and var. *olivacea* found by him in his experiments are not very convincing and

[1] As Prof. P. DRACH has pointed out to me, it is easy to see, if a specimen of *L. obtusata* has completed its growth. In growing individuals the mouth edge is sharp and the inner layer is dull near the mouth, while in full-grown specimens the mouth edge has become rounded and the inner layer is shiny.

Van Dongen (1956) found no difference at all between these two varieties as regards reaction to scent of Fucaceae and food preferences.

IV. THE FOOD PREFERENCE OF *LITTORINA OBTUSATA*

A. INTRODUCTION

In the foregoing chapter it has been shown that *L. obtusata* is, in fact, confined to the belts of four algal species: *F. spiralis*, *A. nodosum*, *F. vesiculosus* and *F. serratus*. When numbers are compared it should be taken into account that differences in numbers may be caused by abiotic factors which govern the environment of the snails in a different way at the respective heights in the tidal zone. However, when differences in numbers are noted on each of the alga species which occupy one and the same or almost the same zone, it is probable that some sort of preference for the seaweeds themselves plays a role.

From the distribution of the snails as found in nature no preferendum can be deduced, so, if we want to study the preference of the snails for the seaweeds, we should offer the algae under experimental conditions. Such preferendum experiments with L. obtusata have been carried out by Barkman (1955).

B. PREFERENDUM EXPERIMENTS

In his preferendum experiments Barkman used a long trough of 200 cm long, 45 cm wide and 25 cm high, in which a constant flow of sea water was maintained. The algae between which the animals could choose were laid down on 8 obliquely placed tiles, while two tiles were kept bare for control (cf. Barkman, 1955, fig. 9).

The algae used by him were the brown *F. spiralis*, *F. vesiculosus*, *F. serratus* and *A. nodosum*, the green *Ulva lactuca* and the red *Chondrus crispus*, *Porphyra laciniata* and *Gigartina stellata*, all at the same time in each experiment. Care has been taken to change the sequence in which the different algae were arranged on the tiles, but it was of course impossible to test all combinations. The experiments were carried out with animals of the var. *citrina*, collected on *F. serratus* and *F. vesiculosus* separately, and with snails of var. *olivacea* from *F. vesiculosus* and *A. nodosum*. The snails were laid down on the bottom of the trough, evenly distributed between the tiles. It appeared that the animals needed 4 to 5 days to make their choice. The results show clearly that there is a strong preference for Fucaceae as compared with the other algae, the ratio Fucaceae: non Fucaceae being 1076: 253.

A preference of the snails for one of the four Fucaceae used, can not be determined satisfactorily from Barkman's figures, although he

made some suggestions. This, however, is not surprising, because the experiments were too complicated. In preferendum experiments all possibilities for choice offered to the animals should be equivalent as regards their availability to each animal. This could not be accomplished in BARKMAN's experiments. Moreover, light and water current appear to influence strongly the direction of the movements of the animals.

In order to eliminate these sources of errors the following arrangement was chosen for the greater part of my own experiments. The aquarium used was about 100 cm long, 75 cm wide and 25 cm high, and it has a rough stone bottom. Black paper had been pasted around the glass walls in order to avoid the influence of light on the choice of the snails, and a hardboard cover had been placed on the aquarium for the same reason and to prevent escape. The aquarium was filled with sea water up to ca 2 cm above the bottom.

Generally, 400 animals were used in the experiments, all collected on the same seaweed species, for there could be a difference in preference between individuals of *L. obtusata* collected on different algae. The snails were arranged on the bottom, evenly distributed between the algae.

The condition of the algae may play an important role in the preferendum experiments, as it may greatly influence their attractiveness. Empirically, it appeared that the same plant should not be used more than two times, because afterwards the process of decaying may bring undesired additional factors into the experimental situation. One day was sufficient for the animals to make their choice.

1. *Experiments with two different algae*

In the first four series of experiments two species of algae were tested at the same time, six tufts of each. These tufts were distributed alternately on the bottom of the aquarium in the manner shown in fig. 3, and their places were interchanged for each new experiment of a series in order to avoid any possible influence of position of the algae on the choice of the snails.

a. Choice between *F. spiralis* and *P. canaliculata*

The first problem to study was the conspicuous absence of *L. obtusata* from the upper algal belt of *P. canaliculata*, while the snails are abundant on the next belt of *F. spiralis* just below. Apart from a number of abiotic factors, a certain preference might be responsible for the upper limit of the vertical distribution of the species.

The first set of experiments were made with 400 animals collected

TABLE VI

Choice between *Pelvetia canaliculata* and *Fucus spiralis*
Exp. 1: 400 animals, collected on *F. spiralis*
Exp. 2: 400 animals, collected on *F. vesiculosus*
Exp. 3: 400 animals, which were collected on *F. spiralis*, and which had been placed for ten days on *P. canaliculata* before the preferendum experiment

	experiment 1		experiment 2	experiment 3	
	1	2	1	1	2
Pelvetia canaliculata	87	86	96	97	92
Fucus spiralis	254	227	230	227	234
Not chosen	59	87	64	47	23
Dead	—	—	10	29	22
Total	400	400	400	400	371

on *F. spiralis*. They had the choice between *F. spiralis* and *P. canaliculata*. Both times the number found on *F. spiralis* was more than twice as high as the number found on *P. canaliculata* (table VI). It might be possible, however, that the experimental animals, coming from the *F. spiralis* belt, had experienced the taste of *P. canaliculata* and that the dislike of this seaweed originated from a process of adaptation. Therefore, the experiment was repeated with 400 snails from the *F. vesiculosus* belt. The result, however, was the same (table VI). To complete the picture, adaptation of 400 snails collected on *F. spiralis* to *P. canaliculata* was tried. Although the animals consumed this seaweed—which was obvious from the large amount of feces produced—it appeared that their preference had not shifted towards *P. canaliculata* in the 10 days during which they had eaten it (table VI).

We are therefore safe in saying that the upper limit of the vertical distribution of *L. obtusata* is correlated with a strong preference for the seaweed just below the limit, as compared with the seaweed just above this limit.

b. Choice between *F. vesiculosus* and *A. nodosum*

The numbers of animals found on *A. nodosum* are always smaller than those found on *F. vesiculosus*, though these two algae occur together just below the *F. spiralis* belt. Some reasons may be put forward to explain this phenomenon. In the first place the *Ascophyllum* plants are generally fixed on top of boulders which lie amidst a vegetation of *F. vesiculosus*. Snails living on the *Ascophyllum* plants may possibly be dislodged rather easily from the long, very smooth fronds by the tidal current and drop into the *Fucus* vegetation around the boulders.

However, here also a preference for *F. vesiculosus* may be the cause of the unequal distribution found in nature. Experiments confirm this

TABLE VII

Choice between *Ascophyllum nodosum* and *Fucus vesiculosus*
Exp. 1: 400 animals, collected on *F. vesiculosus*
Exp. 2: 450 animals, collected on *F. vesiculosus*
Exp. 3: 400 animals, collected on *A. nodosum*

	experiment 1		experiment 2		experiment 3			
	1	2	1	2	1	2	3	4
Ascophyllum nodosum	178	168	150	108	124	151	89	117
Fucus vesiculosus	216	214	286	295	265	213	261	242
Not chosen	6	18	14	47	11	32	34	25
Dead	—	—	—	—	—	4	12	—
Total	400	400	450	450	400	400	396	384

last suggestion. Four hundred animals, collected on *F. vesiculosus*, were given the choice between *A. nodosum* and *F. vesiculosus*. The results of the first set of experiments were not very convincing, though the number of animals on *F. vesiculosus* was higher than the number on *A. nodosum* both times (table VII). A repetition of the same experiment with other animals indicated, that *L. obtusata* has a greater preference for *F. vesiculosus* (table VII).

This implication is reinforced by the following set of experiments, in which 400 animals, collected on *A. nodosum*, were used. Even these snails showed a distinct preference for *F. vesiculosus* (table VII).

So, again, we can safely say that the unequal distribution of *L. obtusata* in the zone where *F. vesiculosus* and *A. nodosum* occur together is correlated with a preference for *F. vesiculosus*.

c. Choice between *F. vesiculosus* and *F. serratus*

In places lower in the tidal zone we find a mixed vegetation of *F. vesiculosus* and *F. serratus*. In these places the numbers of *L. obtusata* found on the two algae do not differ very much in transects B and C on Ile Verte, while in the Bay of Douarnenez 103 snails were found on *F. vesiculosus* and only 13 on *F. serratus*. But, as has been said, the individuals found in the lower zones are almost all juveniles. Full-grown or nearly full-grown snails are much more abundant on the *F. vesiculosus* plants growing higher up.

Experiments with 350 animals, collected on *F. vesiculosus*, showed that there is a clear preference for *F. vesiculosus* when the snails have the choice between *F. vesiculosus* and *F. serratus* (table VIII).

Here, too, it was tried to adapt the snails to *F. serratus*. For that purpose 355 snails, collected on *F. vesiculosus*, were forced to satisfy themselves for 10 days with *F. serratus*. It appeared that this seaweed

TABLE VIII

Choice between *Fucus serratus* and *Fucus vesiculosus*
Exp. 1: 350 animals, collected on *F. vesiculosus*
Exp. 2: 355 animals, which were collected on *F. vesiculosus* and which were placed for ten days on *F. serratus* before the preferendum experiment

	experiment 1				experiment 2	
	1	2	3	4	1	2
Fucus serratus	100	127	101	96	99	122
Fucus vesiculosus	230	185	201	185	208	182
Not chosen	20	38	33	49	38	41
Dead	—	—	15	5	10	—
Total	350	350	350	335	355	345

was consumed eagerly: the fronds were full of holes afterwards, but the preference for *F. vesiculosus* had not changed (table VIII).

d. Choice between *F. vesiculosus* and *F. spiralis*

Summarizing, we can say that full-grown or nearly full-grown individuals of *L. obtusata* from Ile Verte have a preference for *F. spiralis* as compared with *P. canaliculata* and for *F. vesiculosus* as compared with *A. nodosum* or *F. serratus*. It remained to investigate if there was any difference in attraction between the two algae which they obviously prefer most. I therefore collected 400 snails on *F. spiralis* and put them on the bottom of the aquarium with *F. spiralis* and *F. vesiculosus*. Only a slight difference between the numbers on the two algae could be found, which indicates that there is probably little or no preference for either of them (table IX).

TABLE IX

Choice between *F. spiralis* and *F. vesiculosus* by 400 individuals of *L. obtusata*, collected on *F. spiralis*

	1	2
Fucus spiralis	188	177
Fucus vesiculosus	201	185
Not chosen	11	35
Dead	—	3
Total	400	400

2. *Experiments with four algae*

In a last series of preferendum experiments four algae were tested simultaneously. This complicated the experimental technique because

now only three tufts of each species were present in the aquarium.

When all algae are equally attractive and the snails are arranged on the bottom of the aquarium at the places indicated with dots, i.e., in the open spaces between four tufts of algae, we should obtain an ideal distribution, as shown in fig. 4. Therefore, when we use four different species of algae (with a different attractiveness), we must take care

a. to make the sum of the chances of mounting the three tufts of one algal species the same for all four species tested, and

b. to surround each lot of snails by all four algal species.

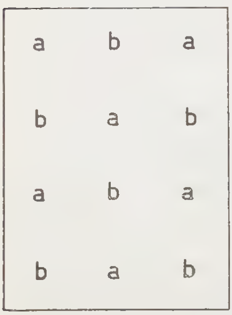

fig. 3

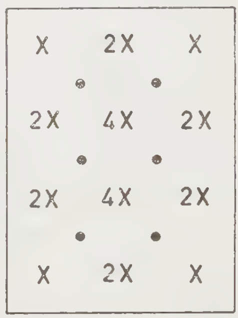

fig. 4

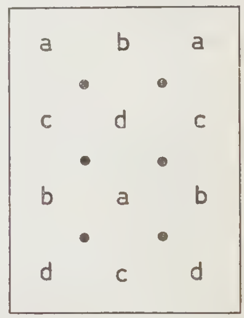

fig. 5

Fig. 3. Arrangement of algae in preferendum experiment with two different algae, a and b.

Fig. 4. Ideal distribution of 24 × animals over the 12 algal tufts, when all algae would be equally attractive. ●: places, where the animals are laid down on the bottom of the aquarium.

Fig. 5. Arrangement of algae in preferendum experiment with four different algae, a, b, c and d.

This can be realized in the way shown in fig. 5. Here the places of the algae were changed also for each experiment.

In these experiments *F. spiralis*, *F. vesiculosus*, *F. serratus* and *A. nodosum* were tested. The first series of 4 experiments I made with 400 animals collected on *F. vesiculosus*. The fifth experiment was made with 450 specimens that had undergone some previous experiments and were not collected on the same seaweed. Table x gives the results.

With the aid of the correlation test for m rankings given by KENDALL (1955) we can calculate the coefficient of concordance, W, for the 5 replicates. We find a value for this coefficient W = 0,904, which means in this case that there is only a very small probability (P = 0,00014) that the differences in numbers of snails on the different algae are caused by chance alone. The ranking given: 1. *F. vesiculosus*, 2. *F. spiralis*, 3. *A. nodosum*, 4. *F. serratus*, is the best estimate of the degree of attractiveness of the algae to the snails.

TABLE X

Choice between four algae
Exp. 1–4 with animals, collected on *F. vesiculosus*
Exp. 5 with 450 animals, that had undergone previous experiments

	exp. 1		exp. 2		exp. 3		exp. 4		exp. 5		exp. 1–5	
	number	%	number	%	number	%	number	%	number	%	number	%
F. serratus	27	7	83	23	40	13	52	19	76	19	278	16
A. nodosum	106	29	78	22	26	8	57	21	98	25	365	21
F. spiralis	117	32	96	26	82	27	78	28	107	27	480	28
F. vesiculosus	120	32	106	29	162	52	89	32	118	30	595	35
	370	100	363	100	310	100	276	100	399	100	1718	100
Not chosen	30		37		51		76		33			
Dead	—		—		39		9		18			
Total	400		400		400		361		450			

It is interesting to note, that BARKMAN (1955) did one experiment, which was very similar to the experiment described here. He used the same four fucoid weeds that were tested by me. Influences of light and water current were excluded. VAN DONGEN (1956) also did four similar experiments, although he maintained a slow flow of water through the basin and used an electric bulb of 60 Watt for illumination. Nevertheless, results of the experiments done by BARKMAN, VAN DONGEN and me can be compared, as is done in table XI.

TABLE XI

Comparison of results of preferendum experiments with four different algae, made by BARKMAN, VAN DONGEN and the present author

	BARKMAN		VAN DONGEN		BAKKER
	citr. %	*oliv.* %	*citr.* %	*oliv.* %	%
F. serratus	46	39	21	17	16
A. nodosum	26	26	16	17	21
F. spiralis	22	26	29	29	28
F. vesiculosus	7	9	34	37	35
Total number of choices	125	97	244	41	1718
Number of experiments	1		4		5

In VAN DONGEN'S experiments the outcome differed enormously for the four separate experiments. In fact, no reliable conclusion can be drawn from them. The results for var. *olivacea*, moreover, are based on a very small number of animals as the author himself admits.

Yet his percentages, based on the total numbers of snails that were found on each seaweed in all four experiments, are not very different from the average percentage that can be calculated from the five separate experiments done by me. The alga that attracted the largest number of snails was *F. vesiculosus*. Next followed *F. spiralis*, then

A. nodosum and *F. serratus*. The result of the single experiment done by BARKMAN is different and can not easily be explained.

It is obvious that these preferendum experiments in which four different algae were tested at the same time, give less convincing results than the experiments in which only two algae were used. All results added together, however, there can be little doubt that *F. vesiculosus* has the greatest attraction, then follows *F. spiralis* and then the other species. In this respect, VAN DONGENG's doubtful figures are substantiated by mine.

3. *Comparison of attraction of two different algae, when snails are placed on one of them*

In his paper BARKMAN mentioned experiments by VERWEY on the perception of the algae by *L. obtusata*. VERWEY found that *L. obtusata* finds the proper algae especially by means of their scent (chemotaxis), and that the snails are able to detect the algae from a distance when a sea water current, passing through the algae, is directed towards them.

These experiments have been repeated more extensively by VAN DONGEN (1956), who found the same results. Attraction caused by Fucaceae at a short distance (as in the preferendum experiments) apparently differs from attraction at a somewhat greater distance; the sequence of attraction at a somewhat greater distance by four species of algae being *F. vesiculosus*, *A. nodosum*, *F. spiralis*, and *F. serratus*. No different reaction was observed between the two varieties, *citrina* and *olivacea*.

I have tried to produce a more complicated situation, in which the animals were placed on an algal species and at the same time were exposed to a water current which had passed through another alga. The problem thus to be investigated was whether the animals would leave the first seaweed and move towards the other one if the latter was more attractive to them.

For that purpose I prepared a narrow aquarium, 120 cm long, 20 cm wide and 25 cm high. Here, too, the walls were pasted over with black paper, and the aquarium was covered with a wooden lid. At one end of the aquarium, just below the sea water tap, a tuft of *F. vesiculosus* was placed and at a distance of 30 cm a thin layer of *P. canaliculata* was spread out. On this layer I placed 200 individuals of *L. obtusata*. The tap was opened to maintain a slow current from the *F. vesiculosus* tuft towards the *P. canaliculata* layer and subsequently towards the outlet at the other end of the aquarium. After two days 112 animals had aggregated on the *F. vesiculosus* tuft; only 17 were left on *P. canaliculata*, while 71 snails could be found on other places. In the reverse experiment, in which *Pelvetia* was placed below the tap and the animals were placed on a layer of *F. vesiculosus*, the result was that 168 snails stayed on *F. vesiculosus* and only 8 had moved towards the

Pelvetia tuft. Twenty-four animals were found elsewhere in the aquarium.

C. CONCLUSIONS

Our final conclusion may be an affirmation of the question posed in the introduction. The numbers of *L. obtusata* on the different algae, as found in nature, coincide with the degree of attractiveness of these algae, as was demonstrated in the preferendum experiments.

Moreover, it seems likely that a certain "dislike barrier" exists, which coincides with the upper limit of the vertical distribution of the species and which is caused by the fact that *F. spiralis* is preferred to *P. canaliculata*. It must be kept in mind, however, that in nature we find *L. obtusata* almost never on *P. canaliculata*, while in the experiments always a rather large proportion of the total number of snails could be found on it. This disagreement is obviously caused by other factors than attractiveness of the algae, and the most likely explanation of this phenomenon is the apparent difference in abiotic environment between the *P. canaliculata* zone and the other zones, which difference was neutralized artificially in the experiments, because here only pure choice was studied.

Another remark has to be made about the obvious fact that a difference in attractiveness shows itself only when two or more algae are present. *L. obtusata* will certainly be attracted by *F. serratus* or *A. nodosum* when no other algae are present, though these algae are less attractive to them when either *F. spiralis* or *F. vesiculosus* are also available.

V. ECOLOGICAL DIFFERENCES BETWEEN *GIBBULA UMBILICALIS* DACOSTA AND *GIBBULA PENNANTI* PHILIPPI

A. NATURAL HABITAT

Fischer-Piette and Gaillard (1956) have described in a brief paper some habits of the two snails. They remark that the two species live largely together, though the habitat of *G. umbilicalis* generally extends somewhat more towards the higher zones. In the lower zones *G. umbilicalis* according to them may be found almost everywhere, while *G. pennanti* is restricted to certain preferred habitats. In tidal pools and in the algal cover of the lower zones *G. pennanti* is much more abundant than *G. umbilicalis*, especially under boulders in the pools. Often, *G. pennanti* leaves its habitat and wanders in large numbers over the neighbouring rocks, though it rarely goes farther away from its "home" than about 50–100 cm. The algae in which *G. pennanti* is most

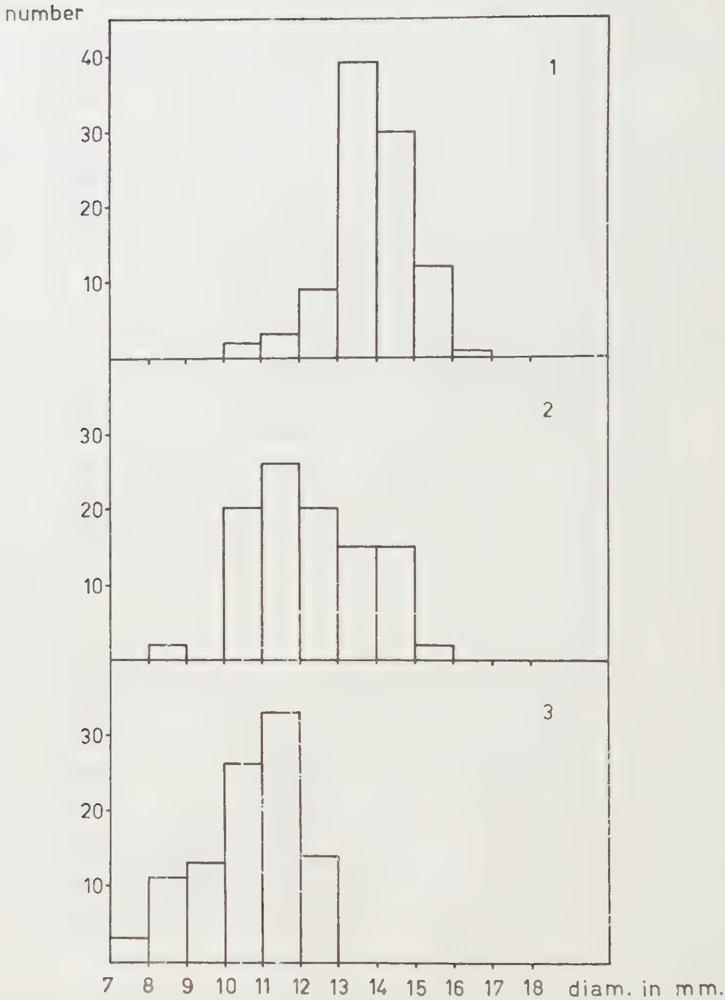

Fig. 6. Distribution of age stages of *G. umbilicalis* over the tidal zone on the Ile Verte, Transect C. 1. sample from the upper part of the *F. vesiculosus* zone; 2. sample from the middle part of the *F. vesiculosus* zone; 3. sample from the mixed zone of *F. vesiculosus* and *F. serratus*, along the *Zostera* bed.

abundant are *Fucus serratus*, *Bifurcaria tuberculata* and also *Himanthalia lorea* when this seaweed is not moved by the waves. This last remark does not agree with my own observations in transect B.

As may be seen from tables I–III, *G. umbilicalis* occupies always higher zones than *G. pennanti*. Their habitats overlap in the lower part of the *F. vesiculosus* zone and in the *F. serratus* zone. Below the latter

G. pennanti is the only one present, its numbers there being extremely high.

We find a peculiar distribution of animals of different ages in *G. umbilicalis:* the older ones live obviously higher up than the juvenile individuals. This is clearly demonstrated in fig. 6. The same phenomenon is observed in *L. obtusata*, where also the young individuals are most abundant in the lowest zones. Unfortunately, the causes of this unequal distribution of age stages remain unknown for the time being, though the strong negative geotaxis in *G. umbilicalis* to be discussed below may be of utmost importance.

Up to now nothing is known about the feeding habits of the two snail species, except for the fact that they are apparently herbivorous. It may be that both of them are essentially microphagous, consuming microscopic algae, lichens, etc., scraped from rocks and large seaweeds with their radulas. Especially *G. umbilicalis* is often seen creeping on the rocks.

Some remarks have to be made on the differences between habits of the two species. In zones where *G. pennanti* was the most obvious species, I observed large numbers of *G. umbilicalis* on loose, bare boulders. Quantitative research in three different places where boulders occur entirely surrounded by one species of alga or by eelgrass showed that the two snails exclude each other to a large degree (table XII). Even the comparatively large number of *G. pennanti* on boulders in the *Zostera* field does not upset these conclusions. The animals had only mounted—which they always do when the tide is out—the lowest parts of the boulders up to just above the surface of the water.

This situation, in which boulders on which one gastropod species lives, lie like islands amidst a vegetation in which the other species of the same genus is almost exclusively found, is a striking example of two "niches", filled by two very closely related species of one genus.

B. NEGATIVE GEOTAXIS

Factors determining the different habitat selection in the two species concerned may be very complicated, as e.g., phototaxis, geotaxis,

TABLE XII

Natural habitat of *G. umbilicalis* and *G. pennanti*

	Coast of the Bay of Douarnenez		Ile Verte, NE of the central rock		Ile Verte, SW of the central rock	
	F. serratus zone		*F. serratus* zone		*Zostera* bed	
	boulders	plants	boulders	plants	boulders	plants
Gibbula umbilicalis	184	7	253	21	449	18
Gibbula pennanti	4	151	15	312	175	411

substratum, food, tolerance of emersion or temperature, etc., in both may play different roles.

Segregation of a mixture of 200 snails of each species was tried by placing them in a large aquarium, 100 cm long, 75 cm wide and 25 cm high. On the bottom *Zostera marina* was spread out in a shallow layer of sea water. Between the *Zostera* leaves 4 small boulders were placed. I surrounded the walls with black paper and in one experiment I closed the aquarium with a hardboard cover in order to avoid the influence of a possible phototaxis. The distribution of the animals was ascertained after a certain time.

Unfortunately, these experiments gave no clear results as may be seen from table XIII. Distinction was made between:

1. animals which had mounted the upper half of the walls,
2. animals which did not mount higher than the lower half of the walls,
3. animals which had mounted the boulders, and
4. animals which had stayed on the *Zostera* leaves.

TABLE XIII

Distribution of 200 individuals of *G. umbilicalis* and 200 individuals of *G. pennanti* in an aquarium

1: upper halves of the walls
2: lower halves of the walls
3: boulders (emersed parts)
4: *Zostera*

Exp. 1: 24 h., dark
Exp. 2: 10 h., light
Exp. 3: 18 h., 13 h. dark and 5 h. light

	experiment 1				experiment 2				experiment 3			
	1	2	3	4	1	2	3	4	1	2	3	4
G. umbilicalis	29	25	77	69	40	29	74	57	37	51	67	45
G. pennanti	13	36	85	66	16	34	64	86	14	43	68	85

There was a difference in the numbers of the two species only in group 1. *G. umbilicalis* was always predominant here, even in the experiment in which light could not have influenced the results, leaving only a negative geotaxis as a possible explanation.

Perhaps this unsatisfactory result is due to the limited height of the boulders. If there is a difference between the species in the intensity of the negative geotaxis (which is suggested by the result of the foregoing experiments) this must show itself more clearly when the animals have the opportunity to mount higher.

For that purpose an aquarium was chosen of 36 cm height, which was divided in 6 zones of 6 cm each, one above the other. The lowest zone was filled with sea water. I put the aquarium in a wooden case, which was painted black on the inside. The upper zone (6) projected above the case. In this aquarium I placed 97 individuals of *G. umbili-*

TABLE XIV

Zonal distribution of 97 *G. umbilicalis* and 101 *G. pennanti* in an aquarium of 36 cm high, divided in 6 zones. First zone filled with sea water, sixth zone projecting above the dark wooden case in which the aquarium was placed. Four experiments.

	exper. 1		exper. 2		exper. 3		exper. 4		total exp. 1–4				
	8 h. dark 8 h. light		1 h. light		4½ h. light		8 h. dark 8 h. light		*G. umb.*		*G. penn.*		
zone	*G.u.*	*G.p.*	*G.u.*	*G.p.*	*G.u.*	*G.p.*	*G.u.*	*G.p.*	tot.	%	tot.	%	tot.
6	29	6	17	3	20	8	32	12	98	77	29	23	127
5	10	10	7	4	10	9	9	4	36	57	27	43	63
4	9	8	17	14	14	12	8	10	48	52	44	48	92
3	10	14	13	20	16	19	13	15	52	43	68	57	120
2	15	39	12	19	17	30	24	28	68	37	116	63	184
1	24	24	31	41	20	23	11	32	86	42	120	58	205
Total	97	101	97	101	97	101	97	101	388	—	404	—	792

calis and 101 of *G. pennanti*, and their numbers in all six zones were ascertained after a certain time. After each experiment all animals were replaced on the bottom of the aquarium.

It was evident that the negative geotaxis in *G. umbilicalis* is much stronger than in *G. pennanti*, as may be seen from table XIV. Though

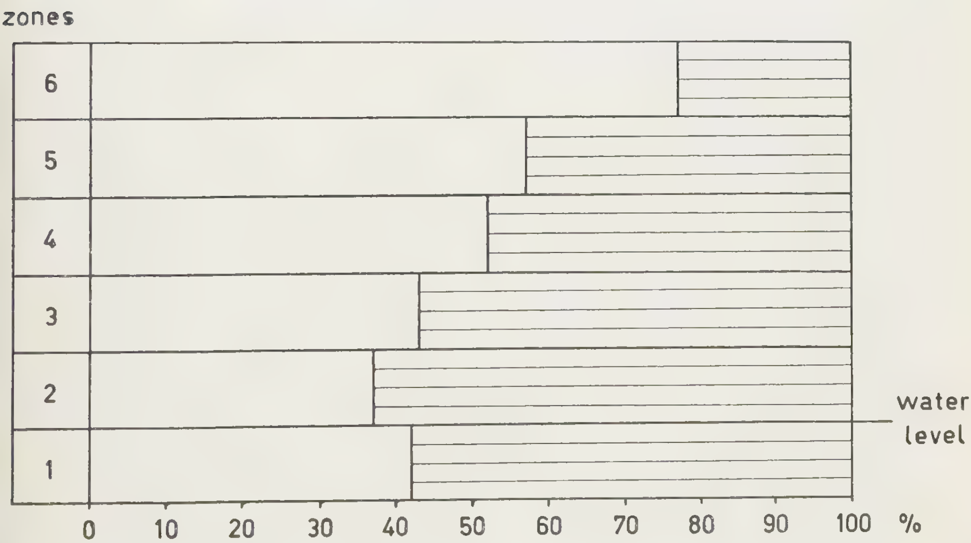

Fig. 7. Negative geotaxis in *G. umbilicalis* and *G. pennanti*. Percentages of the two species in each zone of the aquarium.

G. umbilicalis.

G. pennanti.

the experiments were not of equal duration, and the results therefore not quite equivalent, they have been summarized for the construction of fig. 7, in which the percentage distribution of the two species over the 6 zones of the aquarium is given.

VI. SUMMARY

1. The zonation of a number of snails in relation to algal belts has been investigated on Ile Verte near Roscoff and in a place on the coast of the Bay of Douarnenez, Brittany. A discussion of this zonation is given.

2. The natural habitat of *L. obtusata* appeared to be limited to the belts of *F. spiralis*, *F. vesiculosus*, *A. nodosum* and *F. serratus*. The snails do not enter the *P. canaliculata* zone, and the numbers found on *A. nodosum* are always smaller than those found on *F. vesiculosus*. The numbers collected decrease distinctly in the lower part of the *F. vesiculosus* and *F. serratus* zones, while the proportion of juvenile to adult specimens increases enormously.

3. It is suggested that the juvenile specimens of *L. obtusata* var. *olivacea* are generally yellowish and can not be distinguished from the young of the variety *citrina*. The significance of colour varieties is discussed, but study of the separate colour varieties was deliberately omitted because of the extreme difficulties encountered in distinguishing them satisfactorily.

4. A set of preferendum experiments was undertaken, which resulted in the affirmation that the vertical distribution of *L. obtusata*, as it is found in nature, is correlated with a preference for the seaweeds on which it is found in the largest numbers. *P. canaliculata, A. nodosum* and *F. serratus* appeared to be less attractive to the snails than either *F. spiralis* or *F. vesiculosus*. Between the latter two no significant difference was demonstrated though the figures suggest some difference in favour of *F. vesiculosus*. When the snails were placed for 10 days on either *P. canaliculata* or *F. serratus*, their preference did not shift towards these algae.

5. Individuals of *L. obtusata*, placed on *P. canaliculata*, will leave this weed and assemble on the preferred *F. vesiculosus* when a water current, passing over the latter alga, is directed towards them. In the reverse experiment the animals stay on *F. vesiculosus*.

6. *Gibbula umbilicalis* occupies higher zones in the intertidal belt than *G. pennanti*, its juvenile individuals living lower down than the old ones.

7. *Gibbula umbilicalis* occurs in considerable numbers on bare boulders amidst a vegetation in which only *G. pennanti* is found. The two species thus exclude each other to a large extent.

8. One factor in this difference in habit of the two snails may be negative geotaxis, which is distinctly stronger in *G. umbilicalis*.

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L. TINBERGEN

THE DYNAMICS OF INSECT AND BIRD POPULATIONS IN PINE WOODS

LEIDEN
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FOREWORD

Prof. Dr. Lukas Tinbergen died on September 1, 1955 at the age of thirty-nine. His death was a deep personal loss to his family, friends and colleagues; it was a severe loss also to the study of animal ecology.

Luuk was always primarily an ornithologist. When he was a boy, around 1930, he and his brother Niko started observations on the food and feeding habits of birds of prey and owls. While Niko gradually concentrated on problems of behaviour, Luuk, although maintaining an interest in behaviour studies, mainly devoted himself to developing the ecological aspects of the early investigations. His interest in the effect of a predator on a prey population led to his thorough and extensive thesis on the influence of the sparrow-hawk on passerine birds. This contribution was already characterized by the tendency to describe the ecological relations between animals as much as possible in quantitative terms, a tendency which was to dominate all Tinbergen's further work. He spared neither labour nor time to measure what was measurable, and to make measurable what had hardly seemed to be within reach of quantitative investigation. His analytical mind, combined with his thoroughness and tenacity, made him very well suited for this work.

Tinbergen came to realize that the influence of one species on another can only be studied from the point of view of the population equilibrium of the latter species. Consequently, when he was asked in 1946 to investigate the influence of song birds on insect populations in woods, he incorporated into his working programme a study of the population dynamics of some insects commonly used as prey by titmice in pinewoods. Impressed by the fact that the density of these populations fluctuates around a constant level, he concentrated on the problem of whether the mortality due to birds and parasites depends on the density of the insects in such a way that regulation results.

With regard to this, several investigators have carried out experiments with laboratory models and attempted mathematical descriptions of the results. Luuk Tinbergen saw a possibility to test these models on populations in the field, and for that purpose collected a vast amount of quantitative data from the same area during the early

summers of at least eight consecutive years. These data not only enabled him to check current ideas, but also to contribute to their further development.

Tinbergen did almost his entire field work on populations in an area he learned to know very thoroughly in his boyhood: the environments of the village Hulshorst near Harderwijk. Having started this work as a student, he continued it, first as leader of the "Vogeltrekstation Texel", then as a biologist at the "Institute of Applied Biological Research in the Field (ITBON)", from 1949 as a senior lecturer, and finally from 1954 as professor of descriptive zoology at the University of Groningen. Part of his work was sponsored by the Dutch Organisation for Pure Scientific Research (ZWO). In all phases he had the assistance of interested students who have benefitted from his balanced knowledge and stimulating influence. Several of these pupils have now themselves taken up work, rooted in his methods and line of thoughts.

When Luuk Tinbergen died, the results of his Hulsthorst work, apart from his thesis on the sparrow-hawk, had only been published incompletely in some preliminary papers in Dutch. However, an important manuscript on the relations between the populations of some birds and those of some of their most important prey had reached an advanced stage. In addition, Tinbergen had been working on a theoretical paper on the combined action of birds and parasites on insect populations. He had already completed the calculations for the models discussed and some graphs. Finally, three of the investigations by his students had reached a stage at which they could be rounded off for publication.

Tinbergen's friends and co-workers felt that all possible efforts had to be made to publish as much of his data and ideas as would still be possible. This is how the present volume came into being. It deals with Tinbergen's ecological work only; an attempt to write up his unpublished work on bird migration has also been started.

Detailed account must now be given of the way in which the two papers designed by Tinbergen himself have been finally prepared for publication. Although very different in content, both deal with aspects of Tinbergen's many-sided study on the insects in pine woods. In order to express their fundamental unity, therefore, they are published as parts I and II of one paper titled: *The natural control of insects in pine woods.*

As has been stated, the manuscript of part I, which now bears the subtitle: *Factors influencing the intensity of predation by songbirds*, had nearly attained its final version at the time of Tinbergen's death. He had repeatedly mentioned, however, that he wanted further checks on

his conclusions on a number of points. He was particularly anxious to settle the problem, raised by the 1955 observations, of the general validity of frass production as a measure of caterpillar density. This task has been undertaken by one of us, and the appendix to part I is the outcome. This contains evidence that 1955 was an exceptional year as to climate, and that it was permissible to pool data for all other years of the investigation in order to obtain the best possible estimate of the relation between frass production and density. Further, we knew that TINBERGEN felt that his manuscript needed a final critical revision on many points. This, too, has been done by one of us (DE R.), who carefully scrutinized every point of the argument, referring to the original data as much as possible. As was indeed to be expected, this led to a full confirmation of all TINBERGEN's major conclusions. On some points, however, some tightening of the reasoning, or a regrouping of arguments, seemed imperative to make TINBERGEN's line of thought easier to follow. In these cases, we have modified the text accordingly, always restricting the alterations as much as possible.

Very different was the situation with regard to the second part of the paper, now titled: *Conditions for damping of Nicholson oscillations in parasite host systems*, for which the whole manuscript had to be written from the start. H. KLOMP, aided by H. WOLDA, has accomplished this task, guided by the computations and the diagrams left by TINBERGEN, and by a thorough knowledge, gained in many discussions, of TINBERGEN's ideas. This attempt to reconstruct TINBERGEN's thoughts and arguments has been successful, though of course not equally so on all points. For instance, KLOMP and WOLDA have not in all cases been able to explain the numerical values used by TINBERGEN in his examples, or details of the shape of some of his graphs. In the majority of cases they have inserted without comment the original values used by TINBERGEN, but sometimes they have changed them to simplify the mutual comparison of the models so that the effect of a given factor was more clearly illustrated. In any case, we are confident that the points on which they failed are all of minor importance. Because of his essential and partly independent contributions, KLOMP has been mentioned as co-author in the title of part II.

TINBERGEN's own contributions appropriately form the backbone of this volume. His first paper deals with the measurement of the population density of a number of prey insects of titmice, and of their relative frequencies in the birds' food. From these data it follows that the number of prey of a given type caught, contrary to what one might expect, is not simply proportional to the density of that type. In contrast, the risk of predation for individuals of each type is small when

its density is either low or high, and reaches a maximal value at intermediate densities. Evidence is presented that the increase in risk with increasing density is due to selective learning, the development of a "searching image" for the prey concerned. The decline at still higher densities is thought to be due to the birds beginning to dislike the food. TINBERGEN's main evidence on searching images is based on the prey species *Acantholyda;* further data in support of his views, based on another prey, the adult moths of *Bupalus piniarius*, are to be found in this volume in the paper by TINBERGEN's pupils. J. H. MOOK, L. MOOK, and H. S. HEIKENS.

The applications of the concept of the "searching image" in his ecological work is one illustration of TINBERGEN's lasting interest in problems of behaviour. A further example is the work of his pupil N. PROP, *Protection against birds and parasites in some species of Tenthredinid larvae*, published in this volume. PROP deals with the factors, other than from density, that determine the risk run by a prey: its size, conspicuousness and palatability. His conclusions are based on observations on the behaviour of tits and chaffinches towards sawfly larvae.

On the basis of all these data, TINBERGEN came to the conclusion that birds alone cannot effectively control the population density of the insects. At increasing numbers of the prey, the percentage predation decreases. However, TINBERGEN found that all prey species also suffer from parasites and this led him to consider the possible co-operation of birds and parasites in controlling the insects.

This is the problem of part II of his paper. It asks why the increasing oscillations inherent in NICHOLSON's parasite-host population models do not occur in nature. It is argued that density dependent variations in the host's mortality, such as result from the density dependent variation in the risk due to searching images, may cause a sufficient damping effect. In addition the possibility of damping by other factors is considered.

The last paper of the volume, by P. GLAS on *Factors governing density in the chaffinch (Fringilla coelebs) in different types of wood*, is patterned on a paper of KLUIJVER and TINBERGEN in which these authors present evidence that even in years of great density the population of titmice in mixed woods, the preferred habitat, does not increase beyond a definite value. When the territories prove to be no further compressible, the excess of birds starts to inhabit the less attractive pine woods. GLAS shows that this mechanism also occurs in chaffinches.

Apart from the authors mentioned, dr. H. N. KLUIJVER, mr. P. J. KUYTEN and mr. L. M. SCHOONHOVEN have contributed considerably with criticism and practical help in checking the data. Thus the present volume became possible through the co-operation of many people who

feel themselves in some way or other TINBERGEN's pupils and have tried to give a small service in return for so much they received from him. The preparation of this volume was greatly facilitated by the help we received from mrs. T. P. J. TINBERGEN-FRENSDORF; her stimulating interest and deep knowledge of her husband's work has often been of considerable importance in reconstructing the material. Thanks are due to the "Groninger Universiteitsfonds" for financial support in the publication of this volume.

We hope that this volume will not differ too much from what LUUK TINBERGEN himself would have written and that it will serve, as have his other publications and activities, to promote our basic knowledge in this difficult and important field of animal ecology.

G. P. BAERENDS
L. DE RUITER

LIST OF MAJOR ECOLOGICAL PUBLICATIONS BY L. TINBERGEN

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THE NATURAL CONTROL OF INSECTS IN PINEWOODS

I. FACTORS INFLUENCING THE INTENSITY OF PREDATION BY SONGBIRDS

by

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I. INTRODUCTION

This paper is a contribution to the study of the natural control of animal populations. It deals with the role played by birds in regulating insect numbers.

Although it is obvious that the size of natural populations of animals is regulated, the mechanism of this process is poorly understood. It is evident that abnormally high densities in some way or another cause mortality and emigration to exceed reproduction and immigration. The reverse effect is found when the density falls exceptionally low. Hence, one or more of the factors just mentioned must vary in relation to population density, but precise information about this relation is still very scanty and there is a great need for detailed investigations.

Thus, a first approach to the problem is to measure the rates of mortality, emigration, reproduction and immigration at different densities, and to investigate the mechanisms involved. The mortality factor is complicated because it is the result of a number of different agents. Each of these may have a different relation to population density, and consequently needs to be studied separately. Moreover, the possibility of their interaction must be considered.

Such an analysis should be followed by a more synthetic approach. If all factors involved had been measured, it would be possible to give an exact quantitative description of the whole regulatory system. This, of course, is still far from being realised for populations under natural conditions. Some important questions, however, may be asked beforehand. For instance, it is known that the density of one species is often stabilised at different levels in different habitats. Further, the range of fluctuations is very different for different species and also for the same species in different habitats. These phenomena have important ecological implications. Hence, the question of their causation deserves every attention. The answer may increase our insight into the organisation of communities.

With these general views in mind, we studied the influence of birds on the populations of insects in pinewoods. As VARLEY (1953) has pointed out, woodland insects are favourable objects for the study of natural control, especially because human interference with their habitat is only slight. Moreover, forestry entomologists have accumulated much information about the life histories of tree insects and their parasites. Finally, work on pest control has yielded many data on the trends of population densities from year to year.

In spite of the occurrence of outbreaks, the populations of woodland insects show "restricted fluctuation" (LACK, 1954, see ch. II), which is the effect of a regulating mechanism. As they normally live in very

low densities amidst a superabundant amount of foliage, they present a suggestive example of regulation by factors other than food, presumably by enemies. Hence, they are a promising object for a study of the influence of enemies.

Our first aim was to determine the relation between the depredations by birds and the population density of prey species, so we investigated the food of the birds both qualitatively and quantitatively, their density, and the density as well as the production of insects. The factors controlling the composition of the food proved to be an important key to our problem. These are treated in the present paper. Applying the data obtained to the problem of the regulation of the insect populations, we soon found that for a satisfactory discussion of the latter other agents, particularly parasites, must be taken into account. This led us to develop theoretical considerations of the combined influence of predators and parasites on fluctuations in the density of the insects. In the context of a more general conceptual framework, these considerations will be presented in part II of this paper (this volume, p. 344).

For two reasons our observations have been restricted to pinewoods. Firstly, an environment as homogeneous as possible was required to facilitate the different types of measurements. Secondly, the work was started because of its economic implications, and therefore the choice of pinewood was obvious.

As a satisfactory measurement of consumption by birds is possible only while they are rearing their young, the whole investigation has been confined to the spring and early summer. Consequently only insect species developing in these seasons have been considered.

Finally, many of our data could be used to study a different problem, viz. the influence of food in the ecology of birds. However, a discussion of this point would lie outside the scope of the present paper.

The routine observations on food composition and prey density involved much labour. I wish to express my gratitude to the following persons, who co-operated in this programme during one or more seasons: Miss J. M. Bekker, Mrs. J. C. van Prooije-Nijenhuis, Miss A. Kremer, Miss T. Koevoets, Miss N. Croin Michielsen, Miss H. Heikens, Miss C. S. Duintjer, Mrs. B. H. Kamphuis-Dost, Miss I. van der Burg, H. Veldkamp, G. Prick, N. Prop, D. van der Hey, P. Glas, J. Mook, L. Mook, P. Kuyten, K. H. Postuma and L. Schoonhoven. Mr. Kuyten also made a great part of the counts of faecal pellets in 1953 and 1954, and assisted in other kinds of work. For technical help in the preparation of the field work thanks are due to J. van der Laan and J. Goossen, and for photographic assistance to K. P. van Kempen.

The investigation was started at the "Instituut voor Toegepast Biologisch Onderzoek in de Natuur" at Arnhem (Director Dr. A. D. Voûte), and was continued at the Zoological Laboratory of Groningen University. We are indebted to Dr. Voûte for many suggestions and for the loan of equipment. Ir. A. E. Jurriaanse (Hulshorst) very kindly allowed us to work in the woods of "Leuvenhorst" estate.

II. THE SPECIES STUDIED

The insectivorous birds which inhabit the area studied are, in order of abundance: Chaffinch (*Fringilla coelebs* L.), Great Tit (*Parus major* L.), Crested Tit (*Parus cristatus* L.), Coal Tit (*Parus ater* L.), Blue Tit (*Parus coeruleus* L.) and Jay (*Garrulus glandarius* (L.)). Among these, the Great Tit was studied most intensively by us. In fact, the present paper deals mainly with this species. Comparative observations were made in all other species except the Jay.

Of the insect species only the more important forms will be mentioned here. I have added a brief description of the stage which is mostly found in the food of the birds (generally the full-grown larva). For more detailed information we refer to the handbooks (*e.g.* ESCHERICH, 1931, 1942).

Cacoecia piceana L. (Lep., Tortr.). A typical Tortricid larva, yellow green or olive green, living in webs between one-year-old needles or attached to the young growing shoots.

Bupalus piniarius L. (Lep., Geom.). After emergence the young moths rest for some time on the ground, hanging from the underside of dead twigs or needles. Especially in this stage, they are very vulnerable to bird predators. The main colours shown by the resting moth are brown, yellow, grey and white in a complex pattern.

Ellopia prosapiaria L. (Lep., Geom.). The full-grown larvae have a complicated pattern of brown, yellow and grey, and closely resemble a pine twig which has shed its needles. The underparts are generally a whitish yellow.

Larentia firmata Hb. (syn. *Cidaria firmata* (Hb.)) (Lep., Geom.). The full-grown larva is bluish green with several narrow white longitudinal stripes on its back and sides. The head and a lateral spot on the thorax are a reddish brown. This larva feeds by night and rests by day. In the resting attitude the head is held close to the twig and the body is stretched alongside a needle. To the human eye, the insect is very well concealed in this position.

Larentia obeliscata Hb. (syn. *Cidaria obeliscata* (Hb.)) (Lep., Geom.). Differs from the preceding species in having a green head and wider light longitudinal stripes.

Panolis flammea Schiff. (syn. *P. griseovariegata* Goeze) (Lep., Noct.). The fifth-instar larva is bluish green with a typically disruptive pattern of white longitudinal stripes, which seem to break up the body up into a number of green strips, each about as wide as a pine needle. The dorsal and ventral parts of the larva are separated by a reddish orange line (yellow in the younger instars).

Lymantria monacha L. (Lep., Lymantr.). This big hairy caterpillar has a typical bark or lichen coloration in grey, brown, black and white. It feeds by night and hides by day under thick branches or in crevices of the bark.

Dendrolimus pini L. (Lep., Lasioc.). A big hairy caterpillar in about the same colours as *Lymantria*. Rests on thick branches. Unlike *Lymantria*, it often feeds by day.

Atolmis rubricollis (L.) (syn. *Gnophria rubricollis* L.) (Lep., Arct.). The moths are sometimes taken in great numbers by birds. They are small black Arctiids with a conspicuous red spot on the thorax and orange abdominal segments. The flight is slow.

Acantholyda nemoralis C. G. Thoms. (syn. *Lyda stellata* Christ.) (Hym., Pamph.). This flattened larva has an olive green or yellowish grey ground colour. Its back and its underparts have three dark brown longitudinal lines, which often fuse together where the segments meet. The larva lives in a loose web between twig and needles.

Diprion sertifer Geoffr. (syn. *Lophyrus rufus* Ratz.) (Hym., Tenthr.). The full-grown larva of this sawfly is greyish green with a general blue tinge and a grey-black

pattern on each segment. The head is dark blue-black. This larva lives in dense colonies of 10–60 individuals.

Diprion pini L. (syn. *Lophyrus pini* (L.)) (Hym., Tenthr.). Larva white-yellow, head brown. A very conspicuous colonial form.

Diprion virens Klg. (syn. *Gilpinia virens* (Klg.)) (Hym., Tenthr.). Larva solitary, bright green with some longitudinal stripes in paler green and a sharp white lateral line. Head green.

Diprion frutetorum F. (syn. *Gilpinia frutetorum* (F.)) (Hym., Tenthr.). Larva much like the preceding species, but head brown with black triangle and lateral line pale green instead of white.

Diprion nemoralis Ensl. (Hym., Tenthr.). Larva solitary, conspicuously coloured in grey with black, white and yellow bars.

Diprion simile Htg. (Hym., Tenthr.). Larva solitary, conspicuously coloured. Each segment has many transverse black lines and bright yellow spots near the spiracles.

III. THE AREA OF THE OBSERVATIONS

All observations reported in this paper were made in a plantation of Scots Pine *(Pinus sylvestris)* called "de Zwarte Berg", near Hulshorst, province of Gelderland, the Netherlands. The area of this wood is 63 hectares (approx. 160 acres). It is situated on preglacial sands which are poor in minerals. The trees were about 35 years old, and 11 m high in 1948. Their growth is medium, their density about normal for the age of the trees. Plate IA gives a characteristic view of the wood.

Narrow strips of deciduous trees have been planted along the principal roads (see map, fig. 1), and a few birches are found inside the wood, which nonetheless is an almost pure stand of Scots Pine. A shrub layer is lacking, the litter being covered mainly with moss. Blueberries and *Aera* grass which are characteristic of the better types of pinewood, are scarce, but in some spots *Molinia* occurs.

Fig. 1. Map of the pinewood on the Zwarte Berg, the site of the observations. Scale: 1:5000. ○ = nestbox on tree; □ = nestbox on hide. Hatched: deciduous trees

About 40 nestboxes for hole-breeding birds were placed in this area before 1935. In 1948, 10 glass-backed boxes with observation hides (Plate IB) were added; their number was gradually increased to 19.

IV. METHODS

I. THE MEASUREMENT OF THE DENSITY OF INSECTS

The density of insects living in the crowns was measured in twig samples and by counting the faecal pellets which dropped from the trees. Emerging *Bupalus* moths were counted in sampling areas on the ground. No attempt was made to estimate the density of prey living on the bole and the heavy branches.

Twig sample method. Twig samples were cut with the aid of a ladder and taken to the laboratory in large bags. In this way, the larvae and pupae of a number of important species could be counted, though many winged adults presumably escaped. For technical reasons, spiders were not sampled. Control observations in which white cloths were laid on the ground showed that placing and climbing the ladder did not cause any loss of specimens, except for a few spiders.

When collecting twigs, we tried to obtain a representative sample from the different layers of the crown. This is necessary because twigs exposed to the full sunlight have a much more luxuriant growth than those in more sheltered positions, and therefore may harbour a different number of insects.

As a rule, 8 or 16 trees were sampled in each tit territory. These were situated 40–60 meters from the nest and distributed evenly all round it. Often, but not always, two more trees were sampled in the immediate neighbourhood of the nest. In this way we got a fair sample of the area where the birds did most of their hunting. Generally three or more broods were under observation at the same time, and then an overall figure for density of insects was calculated from the twig samples collected near all these nests. A census of this kind was made when the young were 10–20 days old, as the more important calculations of food consumption all refer to young of this age.

Most of these samples were examined visually only. Each twig was closely scrutinised from different sides, and dense shoots were twisted or bent in order to discover insects which were hidden behind the needles. In the first years a number of samples were examined twice. These duplicate counts showed that some eggs and young larvae had been overlooked, but that the bigger prey (which are the object of this study) had all been found at the first inspection. Further all samples were examined roughly again when the shoots were counted (see below),

Plate I. A. Pinewood on the Zwarte Berg.

B. Close up of an observation nestbox with hide.

but this second inspection very rarely led to the discovery of an overlooked prey.

On the advice of Dr. H. Klomp, some of the samples of 1953 and 1954 were treated with CO_2, and the anaesthetised caterpillars were collected by vigorously shaking the twigs. This method is much less laborious. For free-living larvae it is very useful, but it gives no information on the density of pupae and of web-inhabiting forms. Control experiments showed, for instance, that many larvae of *Acantholyda* and *Cacoecia* did not leave their webs when anaesthetised.

When the insects had been counted, the resulting figures must be related to the size of the area sampled. Two methods have been used to determine this area. In 1947–1949, the number of one-year-old needles in the sample, and, by a laborious procedure described elsewhere (Tinbergen, 1949), the number of these needles per square meter of woodland were ascertained. In 1950–1954 the number of shoots carrying one-year-old needles in the sample was used. This procedure takes much less time and was, therefore, preferable. In the present paper, all density figures are expressed as numbers of animals per 400 of these shoots. It will be shown below that this figure corresponds on the average to 1 square meter of the wood in which we worked.

When, as in the present paper, the reactions of birds to different densities of prey are studied, it is sufficient to express density as number of animals per number of shoots. The searching efforts of the birds depend in the first place on the amount of plant material which they must inspect, and not on the equivalent area of woodland. Suppose, for instance, that a certain species of prey has the same number of individuals per unit of area in different years, but that the number of shoots per unit of area fluctuates from year to year. Then the birds will have more trouble to collect prey of this species when shoots are abundant than when they are scarce.

The number of shoots per unit of area must be determined, however, if one wishes to compare the consumption by the birds with the total number of prey insects present or produced in their hunting ground. Important theoretical conclusions will be based on data of this kind in part II of this paper, and therefore the calculation of the number of shoots per unit of area will be described here.

In principle, this figure has been assessed by multiplying the density of trees by the average number of one-year-old shoots per tree. The number of shoots was counted on felled trees. Two difficulties must be met in this practice: the number of shoots per tree varies greatly, and it is difficult to take a representative sample of trees. We therefore proceeded as follows. Beside the number of shoots, the circumference at the base of each of the felled trees was measured at a constant height

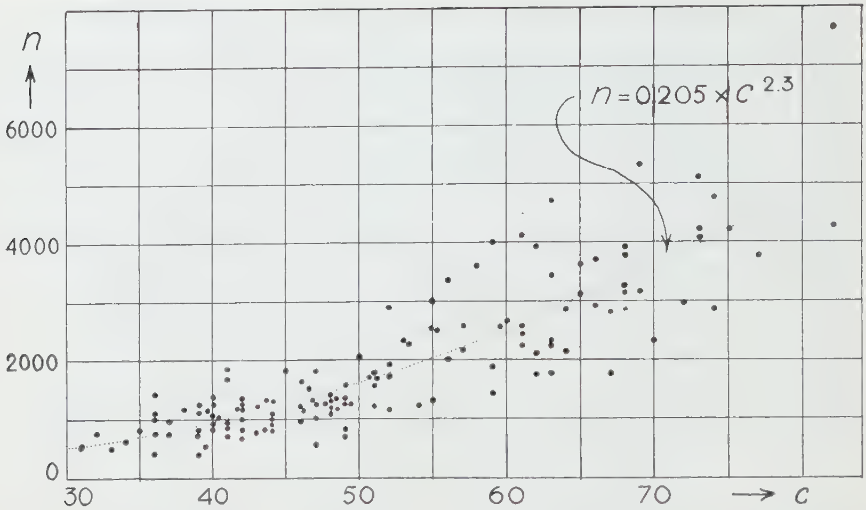


Fig. 2. Relation between number of one year old shoots (*n*) of a tree, and the circumference (*c*) in cm of its trunk at the base

above ground level. A correlation diagram was made of these two quantities, and a curve fitted to the points plotted (fig. 2). This curve can be expressed by an equation of the type

$$n = a \times c^p$$

(in which *n* is the number of shoots per tree, and *c* the circumference of the trunk in centimeters), the best fit being obtained by giving the two constants the following values: $a = 0.205$ and $p = 2.3$.

The final step was to measure the density of trees and the circumference of each tree in 800 areas of 5×5 m each, which were situated in transects across 10 different blocks of the wood. From these data, we were able to calculate the number of shoots in each area, and thus the average number of shoots per square meter, by applying the above formula. The resulting average number of shoots was 400 per sq.m.

As already said, the number of shoots was counted on felled trees. These belonged to three categories: trees cut in ordinary thinnings, trees infested with the fungus *Peridermium*[1], and trees blown down in storms. This practice is open to criticism, since it might bias the sample. It would certainly have been better if a random sample had been drawn from the standing population, but this was impossible for practical reasons. Probably, the resulting error was not serious, as can be seen from a comparison of the needle method and the shoot method. The result of the needle method, being based on random samples of twigs and of falling needles per unit of area, is not

[1] Such trees are cut down as soon as resin is seen on the bole. As a rule, their needles then have not yet died. Specimens with dead parts in their crowns were never used for shoot counts.

biased by overrepresentation of any one of the categories of trees mentioned above. For the summer of 1949, the number of one-year-old needles was calculated as 25900 per sq.m. The average number of needles per one-year-old shoot was 61.5 in the twig samples (10000 shoots from 25 trees). One thus finds 421 shoots per sq.m., which is close to 400. This result suggests that the accuracy of our estimate of the number of shoots per unit of area was sufficient for our purpose, its probable error being rather smaller than that either in the estimate of the number of larvae per shoot, or in that of the percentage composition of the food.

Faecal pellet method. Of the species studied, *Panolis*, *Acantholyda*, *Lymantria*, *Dendrolimus* and *Hyloicus* produce faecal pellets which can be identified by their characteristic shape (ESCHERICH 1931, 1942). In the Geometrids and the *Diprion* larvae, it is not possible to distinguish the species, but each can be separated as a group. The amount of frass produced has often been used for a rough estimate of density, *e.g.*, in checking the efficiency of control measures. On the advice of Dr. VOÛTE, we applied this method also to the measurement of the much lower densities normally found by us.

For this purpose we used frass collectors made of cheese-cloth hanging in an iron frame of 50 × 50 cm. The cloth was stretched and prevented

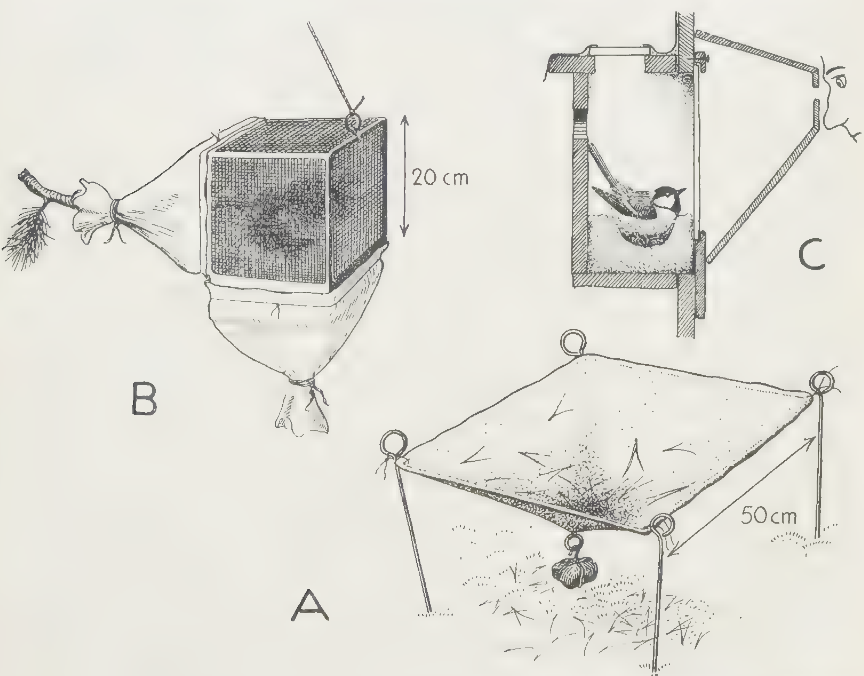


Fig. 3. A. Mounted frass collector. B. Cage for rearing *Panolis* in the trees. C. Diagram of observation nestbox with glass back

from fluttering in the wind by means of a stone. The collectors were supported by four pegs pushed into the ground (fig. 3a).

The collectors were emptied about once a week. Emptying was only possible in dry weather. The contents could be kept for a long time, and were usually examined in the next winter. In order to exclude the young instars, only faecal pellets having a diameter of more than 0.6 mm were counted. The size classes were sorted by sight on a piece of cardboard ruled at 0.6 mm intervals. (We used a reduced photograph of millimeter paper.)

Control observations showed that strong wind and heavy rain (during thunderstorms) did not affect the efficiency of these collectors. Counted samples of faecal pellets (over 0.6 mm in diameter) were scattered over the cloth and collected again after the unfavourable weather. Although these observations took place under extreme conditions, the loss was never greater than 5%, and usually smaller or *nil*. It is possible that very small pellets will sometimes be destroyed in heavy rain. We did not check this, since we were interested only in older larvae.

Errors could also arise if the collectors threw back part of the faecal pellets as they dropped from the trees. In order to examine this influence, we observed the path of pellets dropped from a height of 3 meters. It was found that they hardly jumped up after hitting the cheese-cloth. Moreover, if they jumped up, the slope of the cloth guided them towards the centre of the collector.

On the whole we found it advisable to use at least twenty frass collectors in the area studied. Obviously, this number must depend on density and other properties of the insect species studied.

Originally we applied this technique only to obtain qualitative comparisons of densities at different places in the same period, for we expected a great influence of weather on frass production. However, a rather good correlation proved to exist between frass counts and twig sample counts (fig. 4). Of course, this does not imply that frass production is not affected by weather conditions, but apparently there were no very great differences in the average climate during the sample periods in the various years (see also Appendix). In any case, it seems justified to translate frass figures into values for density of larvae. We have used this in the case of *Panolis* and *Acantholyda*. For these two species, we have employed density figures calculated only from frass counts in a number of cases (marked with a double asterisk in tables II and X; a single asterisk indicates cases in which parallel twig samples and faecal pellet samples were taken).

A more detailed study of frass production in *Panolis* was made by K. H. POSTUMA. For this purpose, *Panolis* caterpillars were reared

in small cages (20 × 20 × 20 cm), mounted in the canopies of living trees in the wood of the Zwarte Berg (fig. 3b). These cages were inspected at short intervals, every day in critical periods. They were placed in two parts of the wood, *viz.* near nestbox 22 and near nestbox 36 (fig. 1). Eight larvae were used in each group of observations, 4 at each of the two sites (2 of these 4 being placed in the lower and 2 in the upper parts of the canopy). Such groups of 8 larvae were installed at regular intervals between June 12th and July 6th. Parallel observations were made in an open tent at ground level.

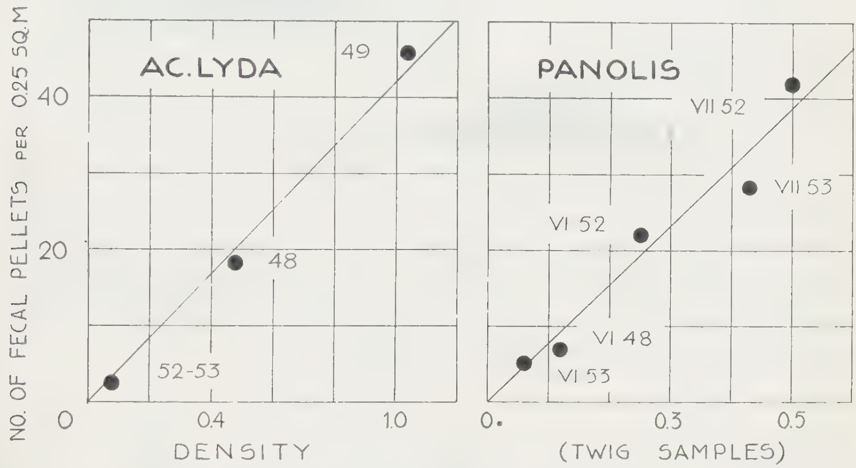


Fig. 4. Relation between frass counts and densities determined in twig samples for *Acantholyda* and *Panolis*. Ordinates: average numbers of faecal pellets (diameter over 0.6 mm) per 0.25 sq. m. per 7 days. Abscissae: density of *Acantholyda* (> 10 mm long) and of *Panolis* (> 20 mm long) expressed as number of specimens per square meter of woodland.

These parallel observations showed that the size of faecal pellets is strictly correlated with the stage of the larva's development. Of all faecal pellets having diameters over 1.2 mm, 95.7 % were produced by fifth instar larvae, whereas these larvae contributed only 3.9 % of the total amount of pellets in the class 0.6–1.2 mm (fig. 5).

In the tree experiments 39 caterpillars produced on the average 340 ± 13.7 large faecal pellets per individual during the fifth instar which lasted 11.7 ± 0.26 days[1]. No differences were found between the groups near box 22 and box 36, or between the high and low groups. Accordingly, the average production per day amounted to 340/11.7 = 29 ± 1.3 large faecal pellets in the fifth instar.

[1] The larvae in the tent produced more pellets than did the larvae reared in the pine trees, but the duration of the 4th and 5th instars was the same in both categories.

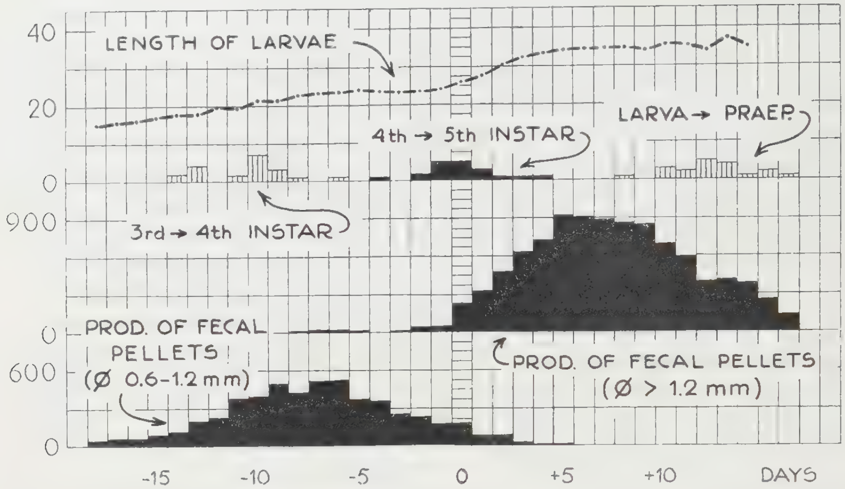


Fig. 5. Production of faecal pellets by *Panolis*. Seven groups of 3 larvae were raised in cages in an open tent. Day 0 for each group: average date on which the members of the group entered the fifth instar. Length of larvae: ordinate, average length of all larvae (in resting attitude) in mm. Moulting dates: ordinate, total number of larvae moulting on a given day to the instar indicated. Frass production: ordinate, total number of pellets (of given size class) produced per day by all larvae. Two larvae died during the observations.

These data formed a link between the twig sample and faecal pellet methods of density determination, which provided a direct check on the density values obtained through twig sampling. Only two series of observations could be used for this purpose, as the numbers of large and small pellets had not been specified in several earlier faecal pellet counts. This led to the following result:

	18–26 June '53	18–29 July '53
number of 5th instar *Panolis* larvae in twig samples	3 on 53.5 sq.m.	15 on 46 sq.m.
expected production of large pellets per 0.25 sq.m.	3.2	26.0
observed production of large pellets per 0.25 sq.m.	3.6	23.1

This close agreement may well be partly due to chance. Nonetheless it suggests that we have not overlooked an important source of error in our technique of density measurement.

The faecal pellet method was used in another way to estimate the density of *Dendrolimus* caterpillars, which are very scarce. For this purpose, 25 frass collectors were erected at equal distances within a square of 5 × 5 m². After two days, a new square was examined, so that in the course of some weeks a considerable area was surveyed. In this way individual larvae could be spotted. P. GLAS has assessed the probability that an individual larva is discovered by this method. In specimens kept

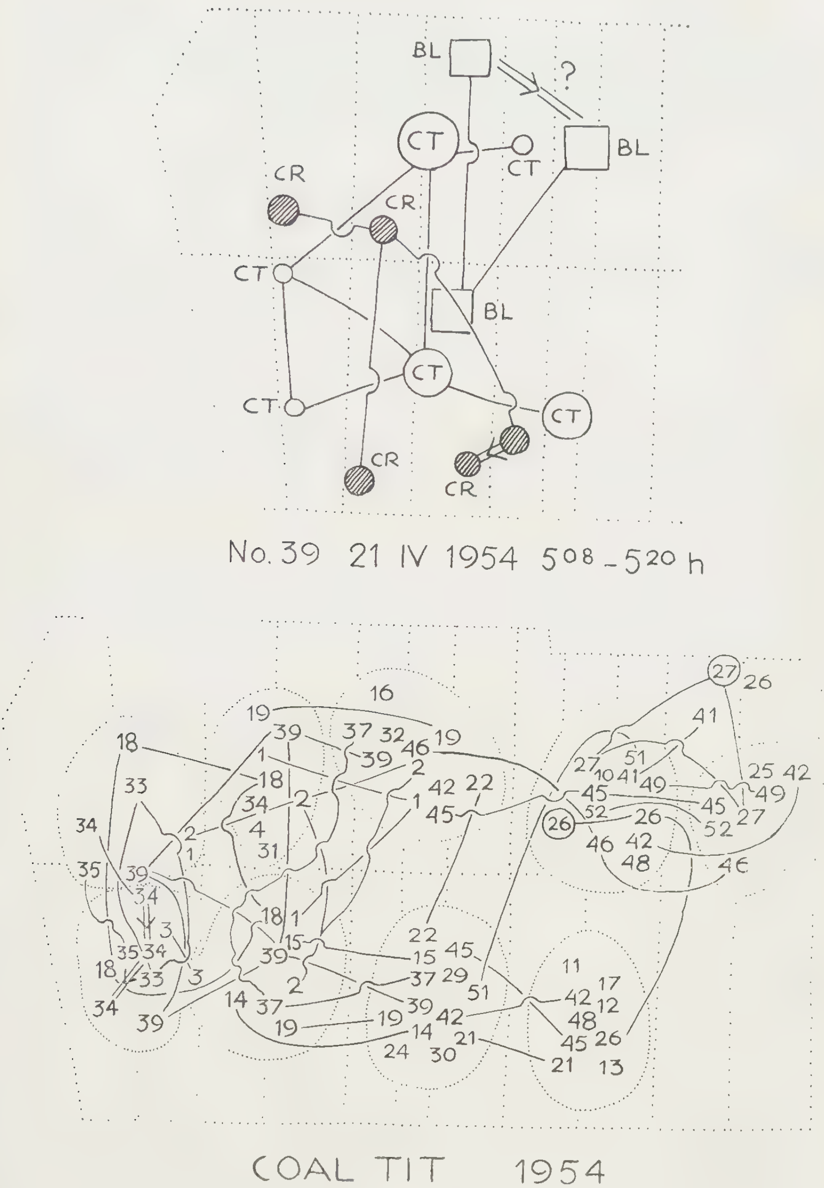


Fig. 6. Census of song birds. A. Specimen of field notes made on one round in the wood. B. Summary of Coal Tit observations in 1954, and their interpretation. Single lines connect individuals heard simultaneously. Double lines indicate displacements of the same individual. Each round is indicated by its number. Small circles denote observations in contradiction with the interpretation adopted. CT = Coal Tit, CR = Crested Tit, BL = Blue Tit.

in cages mounted in the trees, he measured the production of faecal pellets and the ratio of the length of time during which defaecation occurs to that of the periods during which it is stopped (*e.g.* in the moult). This led to the result that the probability of discovery by our method was about 0.5 for big larvae in spring. The density figure for *Dendrolimus* (table 11) was computed in this way.

2. THE MEASUREMENT OF THE DENSITY OF BIRDS

The density of the birds was deduced from the number of occupied territories in April. These were counted during early morning song. The observations took place during 1½ hours per day, from one hour before sunrise onwards. In this time, three to five rounds were made in a certain part of the wood, about 12 hectares in size. The observer cycled along the paths between the blocks. At each round the singing birds were plotted on a separate map. A special symbol was used for males which had been heard simultaneously. Displacements of one and the same male were also recorded. Both kinds of information are important.

This procedure was repeated at intervals of about one week, about four complete counts being made in each part of the wood. Eventually a survey chart was drawn in which all observations on one species were combined. Fig. 6 shows that the different territories can easily be recognised on such charts.

In the Great Tit, which in our area lives only in nestboxes, the results of census counts were repeatedly compared with the numbers of first broods, and a close agreement was found (table 1). In the other species such a check was not possible.

TABLE I

Great Tit. Comparison of counts of territories and first broods

	1950	1951	1952	1953	1954
Number of territories	7	14	15	6	10
Number of first broods	6	13	14[1]	6	10

[1] And one unmated male.

3. OBSERVATIONS ON THE FOOD OF THE BIRDS

Almost all observations on food and feeding frequency were made in glass-backed nestboxes attached to a hide, where the birds could be observed at a distance of about 20 cm (Plate 1B and fig. 3c). This method was suggested to us by the work of PROMPTOV and LUKANA (1938). A roof window in the nestbox provided the required amount of light. A screen with two eye-holes was placed at a distance of 15 cm from the glass back wall. The hide was kept dark inside. These

precautions were sufficient to make nearly all the birds very tame.

The prey brought by a parent was identified by sight. Especially in the first years we also collected samples by the collar method (KLUYVER, 1933). In later years this method was used only to get specimens of prey which we could not identify by sight. For regular observations we abandoned it because the yield is much smaller in tits than in starlings, for which KLUYVER developed the method.

Two circumstances favoured the identification of prey. Firstly, Great Tit and Blue Tit, our most common species, generally bring only one insect at each visit to the nest. Secondly, the number of species in the pinewood fauna is small and most of them can easily be recognised.

We always kept at hand for comparison a collection of live specimens of as many species as possible. Moreover, most of the persons who made the food observations were engaged in the inspection of twig samples as well, and thus refreshed their knowledge of species continually.

The most serious handicap in this work is experienced when the food is passed to the nestlings too quickly. This happens especially when small prey is being fed to large nestlings. Even under more normal conditions, a certain amount of prey escapes identification. We shall explain below (p. 285) how these unidentified items were dealt with in the further calculations.

The length of each prey was also noted; this was done by comparing its size with the length of the bill.

Great Tits and Blue Tits prefer ordinary nestboxes over boxes on hides, presumably because the latter are too close to the ground. For this reason, a normal number of ordinary nestboxes had been left in the wood. The danger was thus avoided that the birds might find the wood insufficiently attractive during the period of settling (*i.e.*, autumn and early spring, *cf.* KLUYVER, 1951; KLUYVER and TINBERGEN, 1953). The entrance holes of the ordinary nest boxes were closed in the beginning of April. The tits which had started nesting in them then moved to the observation boxes; we found that none left the wood.

In the earlier years some birds were allowed to breed in ordinary nestboxes. These broods[1] as well as some broods of Crested Tits in natural sites were also used for food observations. For this purpose a hide was placed at 10 m distance and the food was observed by means of 7 × 50 or 6 × 30 binoculars. This method, which worked only for large kinds of prey, was abandoned in 1950.

[1] In total 3 Great Tit broods and 3 Blue Tit broods.

TABLE IIA

Density of prey species in May, June and July for a number of years. *: density figure based on parallel twig samples and frass counts; **: density figure based on frass counts alone; all other density figures based on twig samples alone. L = larva, P = pupa, Par. L. = parasitized larva. Stage not mentioned: larva. Unless stated otherwise, larval densities refer to specimens longer than 10 mm. Density expressed in number of individuals per 400 shoots = 1 sq.m.

MAY

Year:	1948	1949	1950	1951	1952	1953	1954
Size of sample:	16.2	13.6	37.4	37.1	76.8	—	62.5
Dates:	20–31	16–31	24–31	31–8 VI	10–31	—	22–31
Ellopia L.	0.06	0.07	—	0.13	—		0.03
Ellopia P.	—	—	0.03	—	—		—
Panolis L. > 20 mm	—	—	—	—	0.03		—
Dendrolimus	—	0.07	—	—	—		—
Acantholyda	0.49	0.07	—	—	0.05		—
Syrphid L.	—	—	—	5.2	—		—
Total 1st class	*0.55*	*0.21*	*0.03*	*5.33*	*0.08*	?	*0.03*
Lar. ob. L.	—	—	—	0.08	—		0.10
Lar. ob. P.	—	—	—	0.08	—		0.02
Lar. Par. L.	—	—	—	0.08	—		0.02
Cacoecia L.	—	—	0.03	0.19	0.18		0.13
Cacoecia P.	—	—	—	0.08	0.08		—
Evetria buol. L.	0.06	—	—	—	0.03		—
Dip. vir. + *frut.* L.	0.12	—	—	—	0.01		—
Total 2nd class	*0.18*	*0.0*	*0.03*	*0.51*	*0.30*	?	*0.27*
Lar. firmata	0.25	0.45	0.29	0.43	0.14		0.11
Dip. sertifer	0.62	9.7	0.62	0.27	0.03		—
Total 3rd class	*0.87*	*10.15*	*0.91*	*0.70*	*0.17*	?	*0.11*

V. THE POPULATIONS OF THE PREY SPECIES

A summary of the census counts of twig insects is given in tables II and III. The insect species have been divided into three classes, *viz.* those which are readily taken by birds ("first class food"), secondly those somewhat less readily taken ("second class") and finally those of very small importance ("third class"). For a more precise formulation, see. p. 298. All data about younger instars (which are not taken by the birds) have been omitted from table II. They are on file in the Zoological Laboratory of Groningen University. Finally, a number of species very rarely taken by tits is not mentioned under class 3 for reasons of space.

The table contains most of the data used in the study of the relation between prey density and consumption. Some figures, however, have been combined as an average, although they are used separately in the further calculations.

It should be stressed that tables II and III do not give a complete picture of the food conditions at different times, for they do not include

TABLE IIB

JUNE

Explanation see p. 280.

Year:	1948	1949	1950	1951	1952	1953	1954
Size of sample:	22.8	14.0	46.5	—	52.4	53.7	43.8
Dates:	5–24	7–24	5–13	—	7–14	18–22	24–30
Ellopia L.	—	0.14	—		—	—	—
Panolis L. > 20 mm	*0.13	**0.02	0.04		*0.25	*0.06	*0.05
Acantholyda L.	*0.44	*1.0	0.32		0.02	*0.04	**0.005
Total 1st class	*0.57*	*1.16*	*0.36*	?	*0.27*	*0.10*	*0.06*
Cacoecia L.	0.04	—	—		0.17	—	0.02
Cacoecia P.	—	—	—		0.02	—	—
Dip. vir. + *frut.* L.	1.00	0.50	0.43		0.15	0.65	0.02
Dip. nem. + *sim.* L.	0.13	0.36	0.15		0.08	0.37	—
Total 2nd class	*1.17*	*0.86*	*0.58*	?	*0.42*	*1.02*	*0.04*
Lar. firmata L.	0.40	0.50	0.34		0.19	0.04	0.09
Dip. sertifer L.	—	3.6	1.50		0.02	0.17	0.09
Total 3rd class	*0.40*	*4.1*	*1.84*	?	*0.21*	*0.21*	*0.18*

TABLE IIC

JULY

Explanation see p. 280.

Year:	1948	1949	1950	1951	1952	1953	1954
Size of sample:	—	—	50.0	55.3	56.4	46.5	—
Dates:	—	—	6–20	2–10	8–21	18–27	—
Panolis L. > 20 mm			0.12	0.38	*0.50	*0.43	**0.04
Lymantria L. > 20 mm			—	0.13	—	—	
Acantholyda L.			—	0.14	**0.02	**0.01	**0.04
Dip. vir. Praep.			0.06	—	—	—	
Dip. pini Praep.			0.28	—	—	—	
Total 1st class	?	?	*0.46*	*0.65*	*0.52*	*0.45*	?
Cacoecia L. + P.			—	0.10	—	—	
Dip. vir. + *frut.*			0.22	0.02	0.02	0.13	
Dip. nem. + *sim.*			0.10	0.18	—	0.19	
Total 2nd class	?	?	*0.32*	*0.30*	*0.02*	*0.32*	?
Lar. firmata L.			0.50	0.40	0.12	—	
Semiothisa lit. L.			0.08	—	0.09	0.02	
Dip. sertifer L.			—	0.22	—	—	
Dip. pini L.			0.26	0.20	0.16	—	
Total 3rd class	?	?	*0.84*	*0.82*	*0.37*	*0.02*	?

all species of prey[1], and, moreover, the distinction between first, second and third class species is only crude. Nevertheless, they give a fair idea of the availability of food at different times. For instance, they show that the second half of May offers less food than June and

[1] As already stated, we did not count spiders, winged insects or prey insects living on the boles, the heavy branches and the ground. Among these categories, only emerging *Bupalus* and *Atolmis* were counted in some years. The winter density of *Bupalus* pupae was determined in each year.

TABLE III

Total density of food in different periods. Note: not all prey species have been included, see text.

	1948	1949	1950	1951	1952	1953	1954
First class food							
May	0.55	0.21	0.03	*5.33*	0.08	?	0.03
June	*0.57*	*1.16*	0.36	?	0.27	0.10	0.06
July	lower	lower	*0.46*	0.65	*0.52*	*0.45*	0.08[1]
Second class food							
May	0.18	0.0	0.03	0.51	0.30	?	*0.27*
June	*1.17*	*0.86*	*0.58*	?	*0.42*	*1.02*	0.04
July	lower	lower	0.32	0.30	0.02	0.32	?

[1] Peak in August (*Panolis* very late).

July. This is not altered by the fact that the *Bupalus* moths (not included in the table) begin to emerge at the end of May, for the main period of activity in this species falls in the first half of June.

Twig sampling in April and early May, and many hours of non-quantitative insect collecting between April and August, revealed that the important kinds of food become much more plentiful from April to June and decrease from July to the end of August. In August, *Panolis*, the first generation of *Acantholyda* and *Lymantria* have disappeared, while some species which grow in the autumn are still too small for the birds. (A second food peak occurs in September.)

However, there are important differences between one year and another. The summer peak may occur in June or in July. In 1951 it even was in May, when Syrphid larvae were extremely abundant,

TABLE IV

Peak years of density in different insect species

	1947	1948	1949	1950	1951	1952	1953	1954
Panolis	+				+	+	+	
Lymantria					+	+		
Acantholyda		+	+	+				
Cacoecia	+				+	+	+	+
Larentia obeliscata				+				+
Larentia firmata	+	+	+	+	+	+		
Diprion virens				+				
Diprion frutetorum		+					+	
Diprion sertifer		+	+	+				
Diprion pini	+			+	+		+	
Bupalus[1]			+	+	+			+
Syrphid larvae					+			

[1] According to the yearly censuses of pupae in litter.

and in 1954 it occurred in August. These year-to-year differences are still more marked when one considers the separate species. GIBB (quoted by LACK, 1954, see p. 59) has shown that the peak of caterpillar density in rich oak wood occurred between May 15th and June 9th in 1948–1951. Although the peak in pinewood may be equally early, it is often much later, *e.g.* about the middle of July. It is probable that this is a consistent difference, as assumed by LACK (1954, p. 54), but more observations in the rich woods might well reveal a greater variation in this type of habitat too.

Further, it is important to note that the peak *years* and the low *years* were different in each species of prey. When we combine the census data and the results of non-quantitative collecting, we come to the list of peak years presented in table IV. This will restrict fluctuations in the general availability of food.

VI. THE FEEDING BEHAVIOUR OF TITS

A full description is not needed here, but some points are of special interest for our problem.

Apart from differences in feeding places, the general hunting technique, of Great, Blue, Crested and Coal Tits is very similar. Most probably, they are all guided mainly by visual clues when searching for prey. Beside free-living insects, several hidden forms (like *Evetria buoliana* in its resin-covered tunnels) are eaten. In the case of *Evetria*, the birds fly from some distance to the infected shoots; they evidently recognise the characteristic webs. Insects in flight are rarely taken. Further, a remarkable feature is the speed at which tits move from place to place when collecting food for their young. Apparently they only take prey which they can discover in a very short time.

Yet we believe that there are slight differences in technique between species. It seems that Great and Crested Tits are more inclined to excavate holes in the bark than are Blue and Coal Tits. In summertime, however, this possible difference is not important.

The prey is often prepared before it is passed to the young. Big caterpillars may be decapitated; hairy larvae are often plucked. Moths and butterflies may be brought wingless. The legs of large spiders are often torn off, and their egg cocoons skinned. Despite great individual differences, large prey are more often prepared than small, and young nestlings receive more prepared food than older ones. Large nestlings receive part of the prey alive.

When collecting food for their young, tits of all four species show a remarkable horizontal distribution within their hunting grounds. They do not fly from the nest in random directions, but they generally make a number of successive flights in the same direction. As a rule, however,

they will not return exactly to the spot where the last prey was captured, so that their searching is not thoroughly systematic. Further, having worked a certain part of the hunting grounds for some time, the tits will suddenly shift to another sector, and so on.

Often both partners of a pair hunt near each other. Each flies to the nest as soon as it has found and prepared a prey, and then returns to join its mate. However, the same temporary attachment to a certain part of the hunting ground occurs also when the mates do not feed together.

Similar relations are found in vertical distribution. Tits have a tendency to make a number of consecutive flights to the same layer in the wood. This is seen, for instance, when Great Tits are feeding on emerging *Bupalus* moths (which they take on the ground), and on prey which lives in the crowns.

In addition, there are seasonal differences in vertical distribution. When caterpillars are at their most abundant, all species of tits feed in the crowns of the pines. At other times, their behaviour varies. Coal Tits generally feed a great deal in the crowns during all parts of the year. Great Tits and Crested Tits easily shift to the trunks or to the ground. Blue Tits are on the whole more faithful to the crowns, but they often move from the twigs with needles to the dead branches underneath. This habitat segregation is very marked in winter and often also in July and August, *i.e.*, in the period between the summer and autumn peaks of caterpillar density. In some years it also occurred in May, especially in 1949 and 1953, when food in the crowns was temporarily very scarce. In May 1949, the Great Tits fed on the ground and on the boles; in May and early June 1953, they hunted only on the ground. In the same periods, Crested Tits were often seen on the trunks, whereas Blue Tits and Coal Tits fed in the twigs.

In all other years, the Great Tits spent part of their time on the ground during May and early June. This happened chiefly during the morning hours when *Bupalus* moths emerged. It was also recorded on May 1951, when it occurred in spite of a very high density of Syrphid larvae in the crowns. The Great Tits thus left the crowns also in a period of food abundance, obviously because the ground, too, was rich in food. In 1948 and 1952 this habit was dropped as soon as *Panolis* larvae had developed in the crowns.

On the whole, our observations of these vertical movements support the views of LACK (1954), HARTLEY (1953), and GIBB (1954), who conclude that the specific differences in feeding places are smallest in periods of high food density. It should be mentioned, however, that the separation of feeding sites in winter is not complete. Great Tits and Crested Tits then feed together on the trunks. Coal Tits and Goldcrests in the needles. The last two species have much overlap in food, as stomach analyses showed us. Apparently they can persist although they are dependent on the same stock of winter food. This seems in contradiction with the hypotheses developed by LACK (1954), and requires further study.

The division of labour between ♂ and ♀ tits is well known. In the first 6 days of the nestling period, the ♂ collects a great part of the food for the young. When the young grow up, the share of the ♀ gradually increases. In the second half of the nestling period, the activ-

ity of the sexes is rather variable. In some pairs, ♂ and ♀ bring about the same amount; in others either the ♂ or the ♀ supplies almost all the food.

VII. THE RECORDS OF THE BIRDS' FOOD

In all, 92 broods (67 Great Tits, *Parus major;* 13 Blue Tits, *P. coeruleus;* 7 Crested Tits, *P. cristatus;* 5 Coal Tits, *P. ater*) were studied between 1948 and 1954. The composition of their food was calculated in "percentages by numbers", *i.e.*, the number of individuals of each prey species was expressed as a percentage of the total number of prey individuals fed. Only for pine seed, egg shell and soil, the number of meals was taken instead of the number of pieces.

Part of the food could not be identified. This was due to two circumstances. Firstly, some prey were well observed, but belonged to species unknown to the observers. In the percentage lists, these have been included under "other species". Secondly, in a number of feedings the observer missed all details, for instance because he was writing down the previous meal, or because the bird fed too quickly, or because it was standing with its back towards the observer. Such items may have belonged to species normally identified as well as to the „other species". Therefore, this category was subtracted from the totals before the percentage composition of the food was calculated. As some species are more easily distinguished than others, this procedure might introduce an error, but not a serious one, since the total number of such "misses" was small (3332 out of 52,509 items recorded).

In all broods observed, the composition of the food was calculated for standard periods, *viz.* period I (from hatching day (0) to day 5), period II (day 6-10), period III (day 11–15), period IV (day 16–20), and sometimes, when fledging was very late, period V (day 21–25). These figures have been computed for ♂ and ♀ separately as well as for both partners together. It was only in some Blue Tits and in all Coal and Crested Tits that separate lists could not be given for ♂ and ♀.

The data for each season were plotted on a time graph. Fig. 7 is an example of such a graph. The diagrams for the other seasons are on file in the Zoological Laboratory at Groningen. It will be shown that the differences in food among the four species of tits are only slight, at least in late spring and early summer. Therefore, it is justifiable to make general lists of food composition which include all the data collected in a certain year. Diagrams based on such lists are presented in fig. 8. They give a general impression of the changes in food in the course of seasons and years.

On the whole, the differences between seasons and years reflect differences in the availability of prey species. The successive occurrence

of *Ellopia* caterpillars, *Bupalus* moths, *Panolis* caterpillars, *etc.*, in the course of the season is clearly reflected in the food. Also the year-to-year changes in abundance of the prey species can be traced back in the lists.

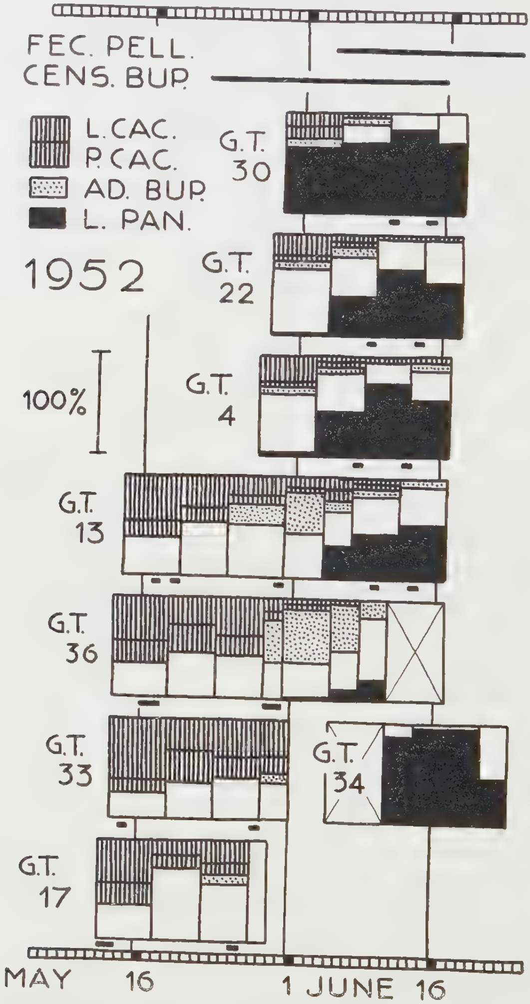


Fig. 7. Time graph of the composition of the food of the first broods of Great Tits in 1952. Percentages in the food for *Cacoecia* (larvae and pupae), *Bupalus* (adults) and *Panolis* (larvae). White: other food. Lines at the top indicate periods during which frass was collected and emerging *Bupalus* moths were counted on the ground. Periods in which twig samples were examined are indicated by lines under the diagrams for each pair. In pairs G.T. 13 and G.T. 36, the young were twice replaced by younger nestlings from elsewhere. Crossed columns: no observations available. The height of each column represents 100% of the food.

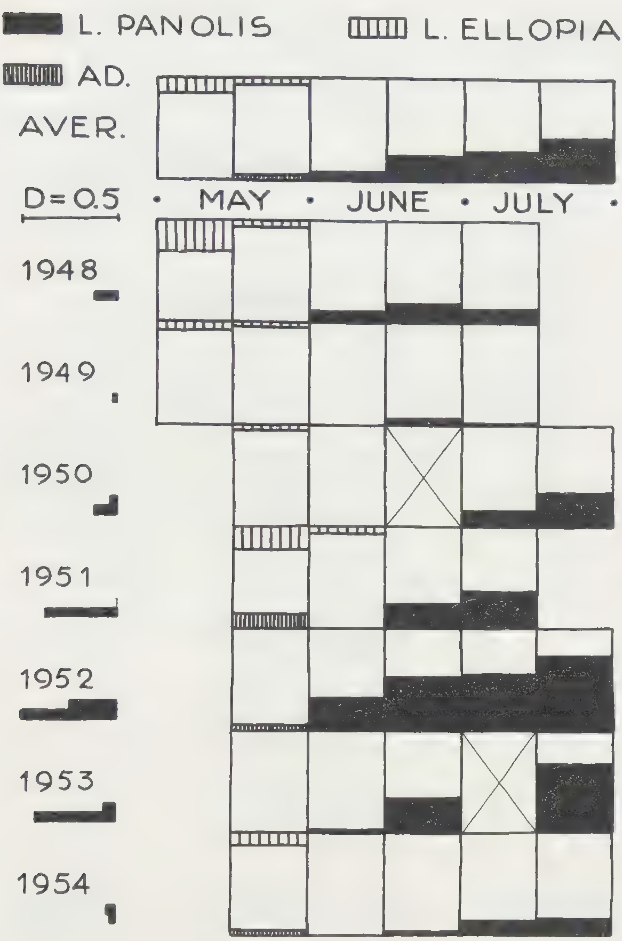


Fig. 8a. Composition of the food of tits (chiefly Great Tit) at the Zwarte Berg in different years. The horizontal bars at the left of the figures denote the density respectively of *Panolis* larvae (fig. a; when two values are given, the upper one refers to June, the lower one to July), *Acantholyda* larvae (fig. b; June) and for *Bupalus* pupae (fig. c; number of pupae per sq. m. in spring). Crossed periods: no observations available.

For instance, there was a very dense population of Syrphid larvae in May 1951 and for some weeks these were the main food, while in all other years they were very scarce.

The detailed examination of these relations is the subject of the remaining sections of this paper.

In this connection it should be mentioned that we sometimes found a marked variation in food which was correlated with the hour of the day. This depended on

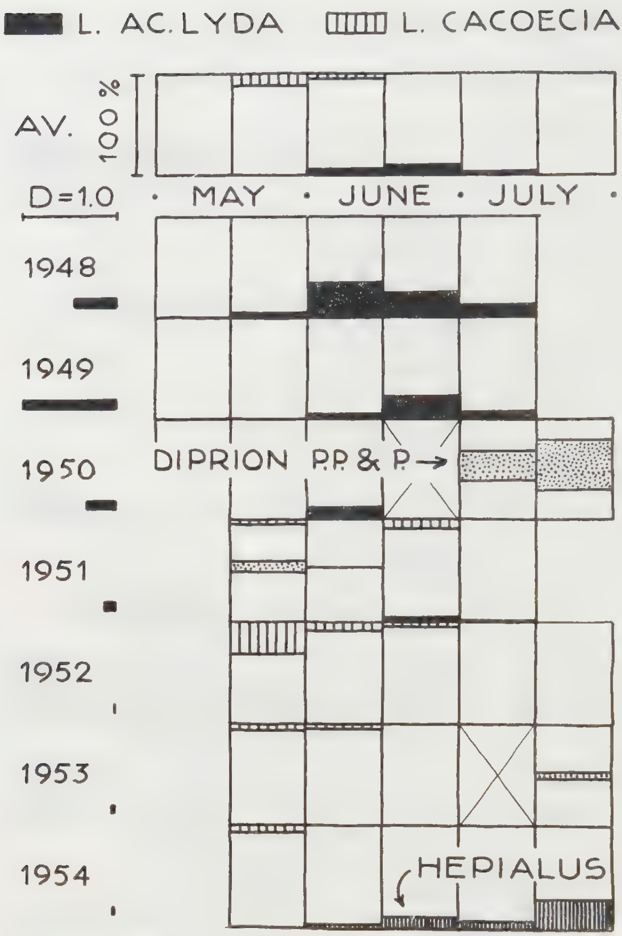


Fig. 8b. Explanation see p. 287.

the diurnal periodicity in the emergence of *Bupalus* moths. The latter event takes place mainly between 7 and 10 a.m. Great Tits often had high percentages of *Bupalus* during these hours and not in the rest of the day. Special care was taken to prevent this influence from biasing the figures used in our further calculations.

A complete list of food percentages is not given here for reasons of space. The more important items can be found in the graphs of fig. 8. One point, however, should be mentioned as it has special interest in connection with the problem of regulation (see pt. II). In three species we have some information on the occurrence of parasitised individuals in the food. In *Larentia obeliscata* it is very easy to distinguish the larvae which have been parasitised by *Litomastix* spec., a polyembryonic

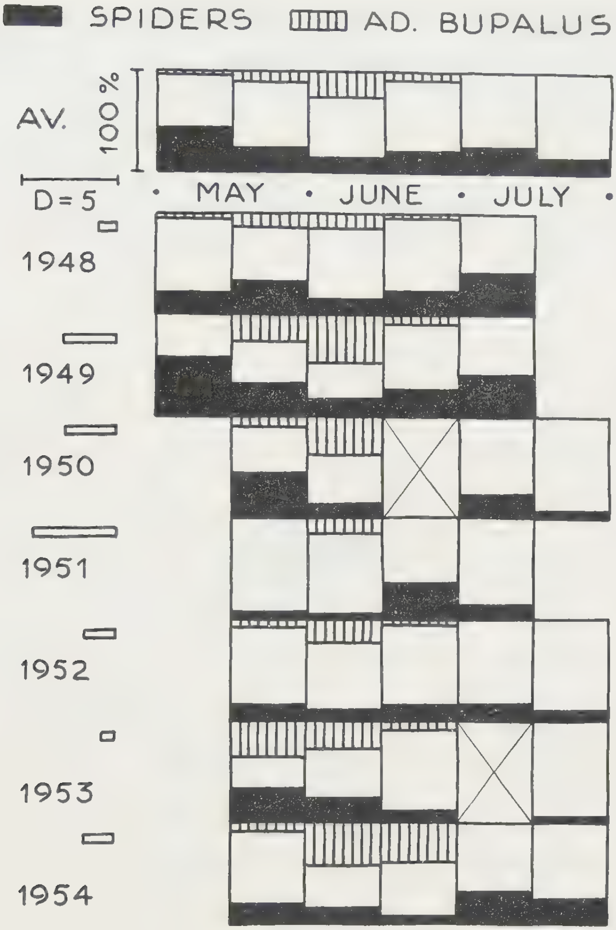


Fig. 8c. Explanation see p. 287.

Chalcid which fills the dried yellowish larval skin with a great number of small pupae. In all, 173 of these infected larvae were observed against 166 normal individuals. *Panolis* caterpillars are infected by Tachinid flies which produce a black scar in the larval skin. Larvae bearing scars were recorded in the food each year, but it is difficult to give an exact figure as the scars are easily overlooked. Finally the records of May 1951 comprise 80 praepupae and pupae of *Diprion pini* and 32 larvae which very closely resembled a parasite larva occurring at the same time in *Diprion* cocoons. Incidental observations in other species also support the view that parasitised insects are regularly taken by Great Tits. On the other hand, we very rarely

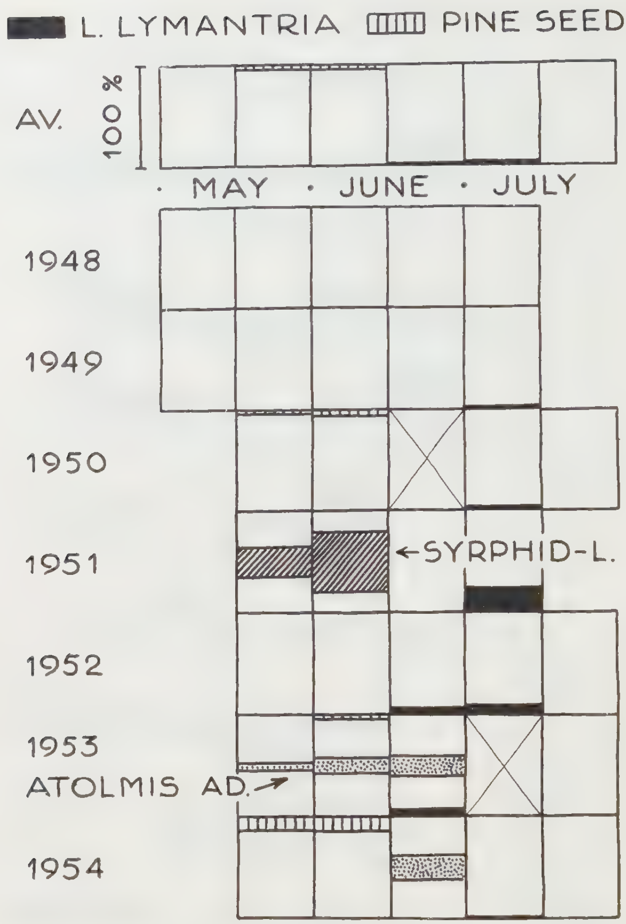


Fig. 8d. Explanation see p. 287.

found adult parasites in the food. The only important exception is mentioned on p. 320.

VIII. THE FOOD OF DIFFERENT SPECIES OF TITS

As already mentioned we found little difference in food between Great, Blue, Crested and Coal Tit. On the whole, the last three species consumed about the same kinds of food in approximately the same proportions as did broods of Great Tits studied in the same year and the same period. Only for the Blue Tit a more detailed comparison can be given. For nine of the thirteen broods examined, parallel observations at Great Tits' broods of about the same age are available.

The composition of the food of these broods was calculated in the usual way over five-day periods. Thus 9 pairs of Blue Tits yielded data on 27 periods. For each of these, parallel figures for Great Tits are available for comparison. When more than one Great Tit pair was available, the average of their food lists has been used. These data have been worked up in two ways. Firstly, correlation graphs were drawn on which for each prey species the percentages found at Blue Tit nests were plotted against those observed at Great Tits nests at the same time (*e.g.*, fig. 9). Secondly the percentages observed in the various five-day periods have been averaged for each prey species, and the averages compared for the two species of tits (table v).

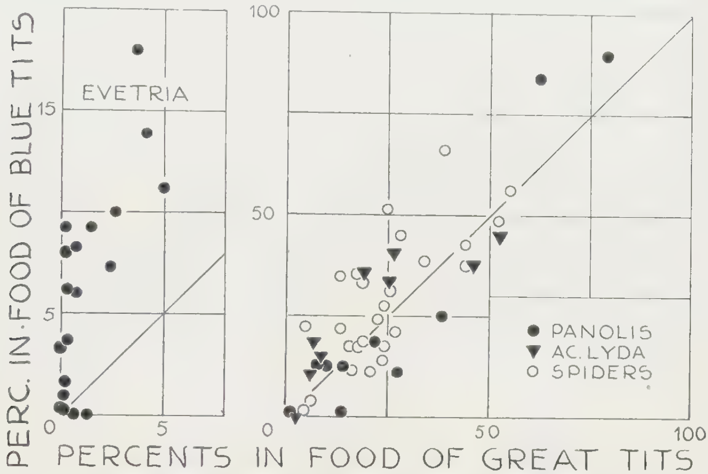


Fig. 9. Comparison of the food of Blue Tit and Great Tit. See text.

It appeared in these ways that the most important difference between the two species is found in *Evetria buoliana*. It is evident that this insect was consistently much more abundant in the diet of the Blue Tit than in that of the Great Tit. A similar but smaller difference is found in *Cacoecia*. Both species live in webs. BETTS (pers. comm.) has found that the Blue Tit is more inclined to collect food which is hidden in webs or galls than the Great Tit. This may be connected with the fact that the former also has a shorter and stouter bill than the latter. The facts reported above agree with BETTS's findings.

In spite of these differences, the overlap in food is very great, at least during May, June and July in the pinewood studied by us. The size of prey is also very similar in the two species. Therefore, as said, some Blue Tit data have been included in the consumption figures discussed in later paragraphs. This is mentioned separately in the respective graphs and tables. It should be stressed, however, that the bulk of our observations refer to Great Tits.

The Crested Tit and the Coal Tit also take roughly the same food as the Great Tit. In these species only seven and five broods, respectively, have been studied by us, so that a more accurate comparison is impossible. However, as a first approximation, an estimate of the total amount of prey taken by all tits together might reasonably be based on the assumption that the food is the same in all four species.

The foregoing also leads to the conclusion that the four species of tits are potential competitors for food in the pinewood studied by us, at least during May–July.

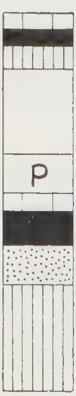
IX. THE PREY INSECTS: THE INFLUENCE ON PREDATION OF SPECIFIC DIFFERENCES IN RISK

There are marked differences in percentage composition between the fauna of the wood and the food of the tits. This implies that the probability of predation varies among prey species. The causes of this variability are examined in the following sections.

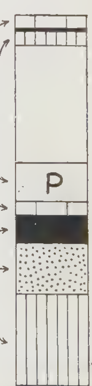
In order to facilitate the discussion, we take as our starting point the following hypothetical, oversimplified model of the relations between predator and prey. Let us assume that each individual of of a prey species A encountered by a given individual predator is captured. The total number N_A of type A prey captured by this predator

TABLE V

Comparison of the food of Blue Tit and Great Tit. See Text



BLUE TIT		GREAT TIT
9	NUMBER OF PAIRS	21
27	NUMB. OF 5 DAYS' PERIODS	27
4056	NUMBER OF PREYS	13388
4.4 %	ELLOPIA PROSAPIARIA	3.5 %
4.3 %	EVETRIA BUOLIANA	0.9 %
6.3 %	CACOECIA PICEANA	3.8 %
9.9 %	PANOLIS FLAMMEA	10.1 %
4.8 %	DIPRION SPECIES	3.6 %
8.9 %	AC. LYDA NEMORALIS	7.2 %
10.0 %	BUPALUS PINIARIUS AD.	12.6 %
28.6 %	SPIDERS	23.4 %
0.2 %	PINE SEEDS	3.6 %
0.1 %	DENDROLIMUS PINI	0.3 %



in a time interval t will then be (assuming random movements of the predator, and random dispersal of prey)

$$N_A = R_A \,.\, D_A \,.\, t$$

where D_A is the population density of species A in the hunting territory during this time[1]. We shall call the proportionality factor R_A the "risk index" of species A for this predator.

Obviously, the value of R_A will depend both on the properties of the predator (*e.g.*, on the size of the area traversed per unit of time), and on those of the prey (*e.g.*, on its conspicuousness, determining how close the predator must come for an "encounter" to take place).

Most of the following sections will devoted to an examination of the factors determining the value of R_A. Anticipating the results of this discussion, we may state that they do not support the simple hypothesis explained above (to which we shall from now on refer as the "probability of encounter hypothesis"). The risk index of a given prey species for a given predator, far from being constant, is greatly dependent on a number of factors, including both the density of this prey species (and of food in general), and learning processes taking place in the predator.

It can easily be seen that the percentage composition of the food is not determined only by the risk indexes of the prey species, but that their *relative* densities will also play a role: all risk indexes being equal, species with higher relative densities would be more strongly represented in the food than those with lower relative densities (for a more precise formulation, see p. 329).

Examples of the influence of relative density will be given on p. 304. However, we shall first present evidence that the value of the risk indexes of the various species depends on several properties of these species.

Clearly the percentage composition of food of the tits is relevant to the problems of regulation which formed the starting point of this study, although more data, *e.g.*, the density of the predators and the duration of the attack, would be needed for a somewhat complete discussion of these problems (see Pt. II).

In connexion with our observations, N. Prop has studied the acceptability of various prey species by the method of choice-experiments. His full results will be published elsewhere (this volume, p. 380), but some of them must be described here as they are indispensable for a better understanding of our subject.

[1] This is true only if D_A is constant during t. Strictly speaking, this does not apply in our case. However, the percentage decrease of D_A during t was rather slight in all cases, so that no great error is introduced if we substitute the arithmetic mean of the values of D_A at times o and t in this equation.

I. THE INFLUENCE ON RISK OF PREY SIZE

It is at once evident that small animals are relatively scarce in the food. In fig. 10, the size distribution of *Acantholyda* larvae found in the twig samples is compared with that of those brought to the nest by a pair of Crested Tits studied at the same time. Table VI presents the size of *Panolis* caterpillars in the twigs and in the food of Great Tits. In both cases, small individuals were abundant in the environment, but scarce in the food. A similar difference has been found in all other species examined.

As far as we could ascertain, the Blue Tit does not take smaller food than the Great Tit, at least during the breeding season. In table VI, some data on a Blue Tit brood observed at the same time as the Great Tits are included. The estimated sizes of *Cacoecia*, brought in by Blue and Great Tits and recorded by the same observer in May 1952, were exactly the same. It is possible, however, that the biggest

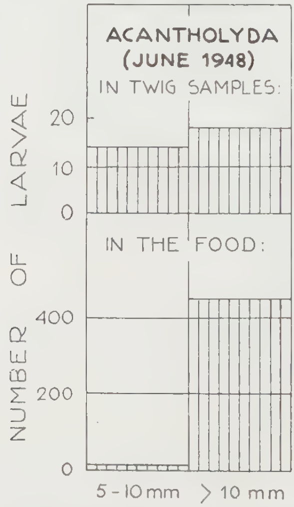


Fig. 10. The size distribution of *Acantholyda* larvae in the food of tits, compared with that in the twig samples. The bigger larvae run a much higher risk.

TABLE VI

The sizes of *Panolis* larvae in twig samples and in the food, July 1952 and July 1953

Instar Length (mm)	III < 18	IV 18–25	V > 25
Number in twig samples	13	23	27
Number in food of Great Tits	0	36	213
Number in food of Blue Tits (1953)	0	1	37

prey insects (*Dendrolimus* and *Lymantria*) are taken in smaller numbers by the Blue Tit than by the Great Tit (*cf.* table v).

The prevalence of large prey in the food may be caused by the tits' preferences, by the fact that large prey are more easily discovered, or by both. In the choice-experiments by N. PROP, a preference for large prey was proved. Although a simultaneous choice will rarely be offered in nature, it is probable that here, too, the preference for large prey is an important factor, *cf.* p. 300.

Three factors complicate the influence of size, *viz.*, the age of the brood, differences between prey species, and individual differences between the parent birds.

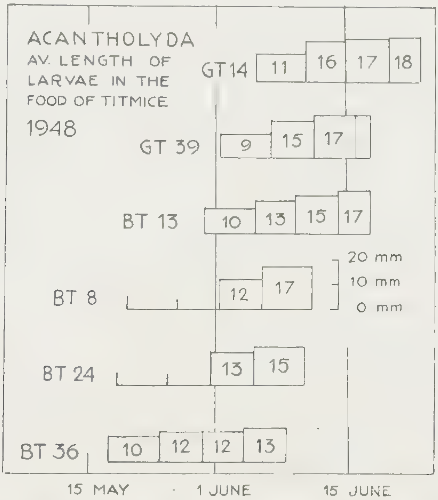


Fig. 11. Influence of the age of the brood on the parents' prey size preference. Height of blocks indicates average length of *Acantholyda* larvae brought to the nest. Figure in each block: average length in mm. Compiled by miss A. KREMER

As for the age of the brood, it was found that the parent birds select larger prey as the nestlings grow older. Fig. 11 presents the average estimated size of *Acantholyda* larvae taken as food in the course of the 1948 breeding season. In the period when some pairs had old nestlings while others had young ones, the latter brought slightly smaller larvae than the former. It follows that selection by the parent tits was involved and that the increase in prey size, found in each of these broods, was not exclusively the effect of growth of the larvae in the environment. Evidently the parents of old nestlings prefer somewhat larger prey than the parents of young ones. The difference, however, is only slight. When large species of prey are present, they are generally brought in considerable numbers, even in the first five days of the nestling period. Only during the first two days the percentage may be very low.

Influences of the environment may even reverse this sequence. For instance, many first broods begin with high percentages for *Ellopia*, which are rather big larvae. This

species pupates during May. It is then replaced in the food by smaller species. This reduces the average size of the prey.

We also have indications that some individual Great Tits regularly selected smaller or bigger prey than others, though the differences were only slight.

As regards differences among species of prey, these have been found to influence the minimum acceptable size. *Panolis* caterpillars, for instance, are rarely brought in sizes under 20 mm, and fifth instar larvae of this species (> 25 mm) run a much higher risk than the fourth instar. On the other hand, *Acantholyda* and *Cacoecia* larvae 12–15 mm long are brought in great numbers, although they are much less heavy than large *Panolis*.

Conversely, it is evident that the preference for certain size classes will also influence the species composition of the food. In fact, a number of small species, which are numerous in the twigs (*e.g.*, *Ocnerostoma piniariella*, *Heringia dodecella*), as well as the young stages of larger prey, are only rarely found in the food. The change in size preference during the nestling period can also be expected to alter the species-composition of the food, the large species becoming slightly more abundant as the young tits grow. Although this influence is only slight, we have elimiated it in our further calculations by basing them only on data obtained during parts III and IV (days 11–20) of the nestling period.

As an effect of size preference, large species are taken by tits when they have grown fat. This seems to be the wise fisherman's policy. It certainly increases the yield as compared with catching all available stages of the same species. It is not certain, however, whether random collecting of small food of all kinds (including those species and instars which now are safe) would also be less profitable. The question is complicated as the tits most probably raise the equilibrium level of population density in the species they prey upon (TINBERGEN, 1955).

2. THE INFLUENCE ON RISK OF SOME OTHER SPECIFIC PROPERTIES OF THE PREY

As already shown, size is not the only factor which determines the risk run by prey species. To explain this we must first present more accurate data on the relations between the species composition of the twig fauna and that of the food.

For this purpose we calculated "indexes of *relative* risk" for a number of species by comparing the risk run by each of these with that run by *Panolis* larvae (over 20 mm long). Thus, if a species *A* is twice as abundant as *Panolis* in the environment, but forms the same percentage of the food taken, its risk is one half of that of *Panolis*.

Such indexes have been computed only for those cases in which both *Panolis* and the other species were present in appreciable numbers.

The advantage of the index of relative risk over absolute values is that the former can be calculated much more easily from food percentages. We have seen that the percentage a species *A* forms in the food depends on the joint influences of its (absolute) risk index and its relative density. Hence, for the calculation of the absolute risk index from a percentage we need data on the densities of all species occurring in the food. However, on the basis of the probability of encounter hypothesis, it can easily be shown that to calculate the relative risk index we need only data on the densities and food-percentages of species *A* and *Panolis* (p. 326).

Of course, it should never be forgotten that the probability of encounter hypothesis is only a crude approximation of the real process (ch. XII). The absolute risk index depends on the density of the species concerned (p. 331). Moreover, it is highly probable that the absolute risk index of any one species will vary with the density of certain other prey species (p. 332). This must always be considered before conclusions are drawn from a relative risk index expressed as a fixed number, independent of the density of either the species concerned, or *Panolis*, or food in general.

Table VII summarizes the figures calculated. Here, as everywhere else in this paper, the relative risk of *Panolis* has arbitrarily been given the value of 100. Before we discuss these, some comments must be made on the method of calculation. (1) The influence of prey size has been partly eliminated. All small stages have been excluded from the density figures, as they are found in the food only very rarely. (2) The consumption figure used is always the average of the percentages

TABLE VII

Indexes of relative risk. The density figures refer to larvae over 20 mm long in *Panolis* and *Lymantria*, over 10 mm long in all other species, and to moths emerging between 7 and 10 h. a.m. in *Bupalus*

Density		% in food		Species	Risk index
spec.	Pan.	spec.	Pan.		
0.125	0.36	23.6	30.1	*Lymantria* > 20 mm	225
—	—	—	—	*Panolis* > 20 mm	100
0.027	0.25	6.2	62.5	*Bupalus*	95
0.29	0.25	18.6	32.8	*Acantholyda*	49
0.17	0.25	3.8	56.5	*Cacoecia*	10
0.35	0.28	0.16	46.3	*Lar. firmata*	0.3
0.156	0.199	0.84	34.3	*Dip. nemoralis* / *Dip. simile*	3.1
0.315	0.199	1.53	34.3	*Dip. virens* / *Dip. frutetor.*	2.2
0.21	0.34	0.03	40.5	*Dip. pini*	0.1
0.16	0.16	0.02	26.9	*Dip. sertifer*	0.1
0.57	0.13	35.4	31.4	*Dip. pini* praep. / pupae	22
0.46	0.13	13.9	23.7	*Dip. vir.* praep. / pupae	16

found in the food of the different pairs under observation. (3) The indexes are based on the average of the individual density values for different periods, not on the total number of animals divided by the total area examined. This was done because the latter figure sometimes gives a wrong impression, especially when the size of the twig samples varied greatly from one period to another.

As Table VII shows, there are very great differences among prey species, the highest risk index being more than 1000 times the lowest. These relative risk indexes are the foundation of the classification of prey species discussed on p. 280: Class I includes all species with a relative risk higher than 15, Class II consists of those ranging from 1 to 15, and Class III is formed by the species with a relative risk less than 1. We shall now discuss the possible causes of the variation in relative risk for a number of the species mentioned in table VII.

Lymantria monacha. The caterpillar has the colour of bark and hides by day under thick branches and in crevices. In spite of being so cryptic, it runs at least as high a risk as large *Panolis* larvae, perhaps because it is so big (up to 6 cm long).

Panolis flammea. Despite a disruptive, more or less concealing coloration the risk of this larva is high. Again this is correlated with large size (length up to 4 cm). As already shown, the younger instars are almost neglected by the tits. Even individuals of 2–2.5 cm length run a much smaller risk than those of 2.6–4.0 cm.

Bupalus piniarius. The moths are taken from the ground when they rest just after emerging. As they are not very big, their high risk cannot depend on their size. It may be caused by the fact that the tits can inspect a certain area of ground much more quickly than the equivalent amount of foliage. We cannot fully discuss here the rather complicated problems raised by this species (see the paper by J. H. Mook and others on p. 448 of this volume).

Acantholyda nemoralis. This web-forming larva is readily detected by the human eye. Its risk is lower that than of *Panolis;* its size is correspondingly smaller.

Cacoecia piceana. The larva is about the same size and colour as *Acantholyda*. It lives in webs and it is about equally conspicuous to the human observer as the latter species. Nevertheless, for reasons unknown, its risk is lower than that of *Acantholyda*.

Larentia firmata. This looper has a highly cryptic coloration and behaviour (p. 268). Although it grows to about 25 mm long, its weight remains rather low. We must suppose that its risk is small through the combined influence of cryptic properties and small size.

Diprion spec. div. These larvae show remarkable differences both in risk and in properties. The two colonial species *D. pini* and *D. sertifer*,

although very conspicuous to the human eye, run and extremely low risk. As size cannot be responsible, they may be protected by low palatability. In fact, they have a sharp resinous taste. As soon as *D. pini* larvae have spun their cocoons, their risk increases about a hundredfold. The tits open the cocoons and collect the praepupae and pupae in great numbers. At this stage the resinous taste has disappeared. This supports the hypothesis that pini larvae are protected by unpalability.

The larvae of four solitary species are taken in greater numbers than those of *D.sertifer* and *D. pini*. The difference is significant (*cf.* table VIII A). Among these four species D. *frutetorum* and *D. virens* are green and more or less cryptically coloured, whereas *D. nemoralis* and

TABLE VIII

Occurrence of *Diprion* larvae (> 10 mm long) in the trees and in the food of Great Tits

A		*pini* and *sertifer*		*frutetorum, virens, nemoralis,* and *simile*
June, July	Number in food	3		48
	Number in twig samples	58		74
			P < 0,01	

B		*frutetorum* and *virens*		*nemoralis* and *simile*
June	Number in food	132		19
	Number in twig samples	94		39
			P < 0,01	
July	Number in food	14		47
	Number in twig samples	19		24
			P < 0,01	
June,	Average length in twig samples	15,4		15,5
July,	Average length in twig samples	16,1		18,7
			P < 0,05	

D. simile have a rather conspicuous pattern of grey, black, white and yellow. In the calculation of risk indexes the two green species have been taken together as one group and the two conspicuous ones as another. This was necessary because of errors of identification in the earlier years of the investigation. In table VII, the two conspicuous species show a somewhat higher risk index than the two green ones. A more detailed analysis is given in table VIII[B]. In June the green species prove to run a higher risk than the conspicuous ones, but the reverse holds true for July.

Table VIII[B] also permits an estimate of the statistical reliability of the data. In both June and July the ratio between green and conspicuous species in the food is signifi-

cantly different from the ratio in the twig samples. Now, by comparison with the inconspicuous species, the conspicuous ones form a small proportion of the food during June, and a large one during July. Hence, the change in risk is also significant.

This increase in risk of the conspicuous species is correlated with an increase in size. The average length of all individuals collected in twig samples is equal for both species-pairs in June, but in July it is 16.1 mm for the green species and 18.7 mm for the conspicuous ones. The difference is significant.

In other words, when both categories were of the same size, the green species were more intensively preyed upon, but when the size of the conspicuous species increased their risk rose over that of the green ones. This again argues for an influence of size on risk (p. 294).

As compared with *Panolis*, the four solitary species run a low risk. This might be an influence of size, but their risk is also much lower than that of *Acantholyda*, which is about equally small. In the green species this might be caused by cryptic properties. For the conspicuous ones, however, this cannot be assumed. Hence, we suppose that the latter, like *D. sertifer* and *D. pini*, are protected by unpalatability or other unattractive properties, although to a smaller extent than these colonial species. As for the green species, it is also possible that these are protected by the combined influence of cryptic coloration and moderately unattractive properties.

Other species. Some small species, which are abundant in the twig fauna but very scarce in the food, have been mentioned on p. 296. *Dendrolimus pini*, *Ellopia prosapiaria* and Syrphid larvae will be considered on p. 303. For the other species on the food lists we cannot give a risk calculation as we do not have reliable figures for their density.

To sum up, the comparison of indexes of relative risk leads to three conclusions:

1. Size of prey is once more proved to have an important influence on risk. This is reasonable because a large prey will be more conspicuous than a small one. On the other hand, the fact that the highly cryptic larva of *Lymantria monacha* runs a very high risk suggests that size also works in a different way. It could be supposed that the preference for large prey (which Prop was able to prove) induces the birds to search intensively for big species once these have been discovered in the area. This would imply a more complicated reaction.

2. Among highly cryptic species the big larva of *Lymantria monacha* runs a great risk and the much smaller *Larentia firmata* an extremely small one. Both are very difficult to find for the human observer. Among moderately concealed species, the third instar larva of *Panolis*

has a very low, and the much bigger fifth instar of the same species a high figure for risk. *Diprion frutetorum* and *D. virens* are also moderately concealed to the human eye. Yet they run a higher risk than four conspicuously coloured members of the same genus, at least during June. In other words, there is no simple relation between cryptic properties and risk. This must not be taken to imply that cryptic species do not derive a certain measure of protection from their patterns. However, no conclusion as to the extent of this protection will be possible until a more analytical study has been made. In any case, the foregoing shows that tits are able to catch both highly and moderately cryptic species in great numbers.

3. In all probability, the low risk of the four *Diprion* species that are conspicuous to the human eye will, on further analysis, prove to be due to unpalatability, or some other unattractive property. All four are supposed to be typical aposematic forms, and two of them (*D. sertifer* and *D. pini*) are colonial.

3. THE INFLUENCE ON RISK OF THE DENSITY OF A PREY SPECIES

Although a full discussion of this phenomenon is better postponed till later, (see p. 331), it must be briefly mentioned here, since it is clearly manifested in figs. 12 and 13. The graphs representing the relationship between density and percentage in the food for *Panolis*,

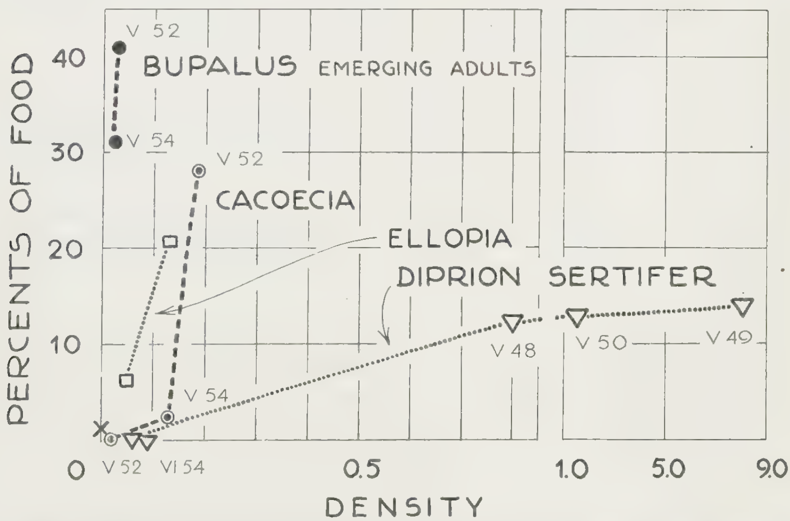


Fig. 12. Percentage in the food and density for several prey species before the appearance of big *Panolis* larvae. Note the change in density scale. The cross refers to *Dendrolimus pini*. Roman and arabic numerals denote month and year, respectively. Only individuals over 10 mm long are included in the density measurements

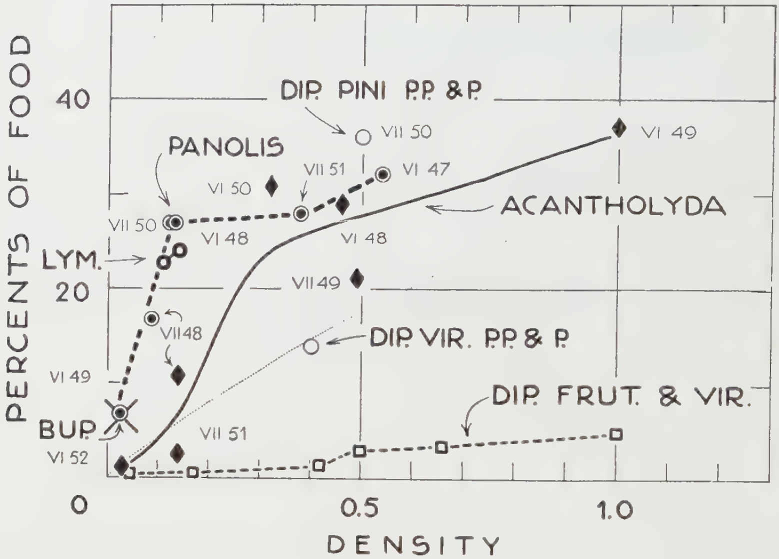


fig. 13a

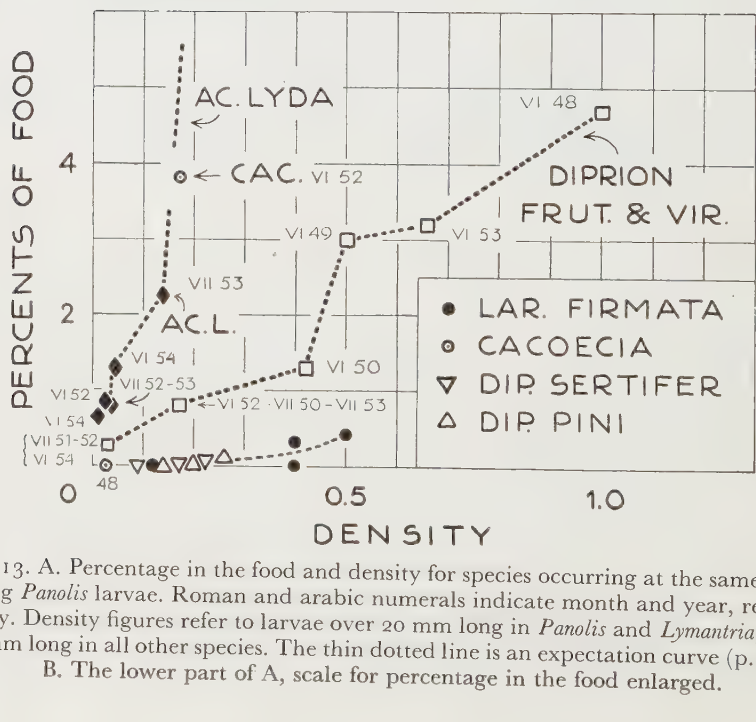


Fig. 13. A. Percentage in the food and density for species occurring at the same time as big *Panolis* larvae. Roman and arabic numerals indicate month and year, respectively. Density figures refer to larvae over 20 mm long in *Panolis* and *Lymantria*, over 10 mm long in all other species. The thin dotted line is an expectation curve (p. 326).

B. The lower part of A, scale for percentage in the food enlarged.

Acantholyda (fig. 13) and *Diprion sertifer* (fig. 12) level off at higher densities.

It would seem that the flattening of the curves might be caused by a decrease in the density of the species concerned relative to that of food in general, or in other words, to an increase in the general abundance of food, to which the species concerned did not proportionally contribute. However, we shall see on p. 329 that this was not so.

This means that, as already said, the value of the relative risk index we find for a given species will depend on the density during the observation both in this species itself and of *Panolis*. For instance, the curves in fig. 13 suggest an increase in the relative risk of *Acantholyda* from about 25 at density 0.1 to almost 100 at density 0.5[1].

This is an example of a probably general tendency for differences in risk between species to disappear at higher density levels, no single species as a rule contributing more than between 30 and 40 per cent. of the food, as long as an alternative is available.

It follows that the indexes of relative risk for various species, in order to be strictly comparable, should all be determined at one and the same density level for all species involved, including *Panolis*. This level should be as nearly normal as possible for all species. Even then it would be necessary to bear in mind that comparisons at other densities would give different results.

For obvious practical reasons, it was impossible in the present investigation to carry out all measurements of relative risk at one and the same density level. We therefore must content ourselves with determinations at densities varying from species to species. Nearly always, however, relative risk indexes have only been assessed at relatively low densities. As a rule, these are the more normal densities, and the differences between species are not obscured by the flattening at higher densities of the density vs. food-percentage graphs.

In the light of the above, it will be clear that, as already said, our indexes of relative risk have only approximative value, and that we should be very careful when basing conclusions on these data. Yet, giving due attention to the precautions thus necessitated, we may derive some additional information on risk differences between species from fig. 12. This is summed up here.

Dendrolimus pini. This large caterpillar much resembles *Lymantria* but is less cryptic (p. 268). At the very low density of about 0.0027, the percentage in the food amounted to 1,07 (average for 1949, 1950 and 1951). This suggests a rather high index of relative risk, comparable to that of *Panolis* and *Lymantria* or even higher.

Ellopia prosapiaria. This species is not very cryptic when feeding

on needles. It is rather big, up to 25 mm in length, and heavier than, *e.g.*, *Larentia firmata*. The figures suggest a risk index between those of *Bupalus* and *Cacoecia*, but this may be an overestimate, as some of the caterpillars may have been overlooked in the counts because they had moved to the branches or the boles during the period of twig sampling (some individuals pupate outside the twigs).

Syrphid larvae. The only figure available is difficult to interpret because of the high density involved. Anyhow the risk index cannot be very low.

[1] These values for relative risk are, of course, very unreliable, as we here disregard the precaution of calculating the relative risk index only from data collected in the same period.

X. THE PREY INSECTS: THE INFLUENCE ON PREDATION OF GENERAL ABUNDANCE OF FOOD

1. YEAR-TO-YEAR FLUCTUATIONS

We have seen that the percentage which a particular species forms in the birds' food depends on the relative density of this species as well as on its risk index. Thus, in a generally rich year, one would expect to find low food-percentages for insect species whose density has remained at a normal level.

In fact, our data revealed a number of examples of this kind. For instance, *Cacoecia* was about equally numerous in 1951 (density 0.19) and 1952 (density 0.23). Now 1951 was extremely rich in food during May, whereas 1952 was about average. The percentage of *Cacoecia* showed a huge difference: in 1951, it was 0.4%, in 1952 27.8%. A similar difference was found in *Bupalus* between 1951 and 1952.

In some of the less attractive kinds of food, this influence seems to be even greater. Pine seeds, for instance, were brought in appreciable numbers during the second half of May 1950, 1953, and especially 1954. In all three years this period was poor in insect food. The pine seed crop was moderate in 1950, very poor in 1953, and poor in 1954. In other words, the higher consumption of pine seed cannot be caused by unusual abundance of this type of food. The adults of *Hepialus hecta L.* were abundant in several years. In the food, however, they were rare except in July 1954, when less total prey was available than in 1948–1953. Finally, spiders most probably show the same effect. These were scarce in the food in May 1951, July 1951, July 1952 and July 1953. All four periods had a higher total density of prey than normal years, whereas spiders seemed to be only normally abundant.

This influence of general food density is not restricted to some exceptional seasons. It will always be at work, and it must be taken into account whenever we analyse the relation between density and

percentage in the food for a given insect species. Accordingly, we have tried to eliminate this influence as much as possible. Within one year, the effect of changes in the abundance of food can be eliminated by treating data obtained before and after the appearance of large *Panolis* separately, as we have done in figs. 12, 13 and 21. Furthermore, in order to obviate the effects of differences between years, the data for *Cacoecia* and *Bupalus* in May 1951 have been omitted from fig. 13, since this month was extraordinary (*cf.* table III).

A further example of the elimination of the influence of fluctuations in the availability of food is to be found in fig. 14, which represents the relationship between density and percentage in the food for *Panolis*.

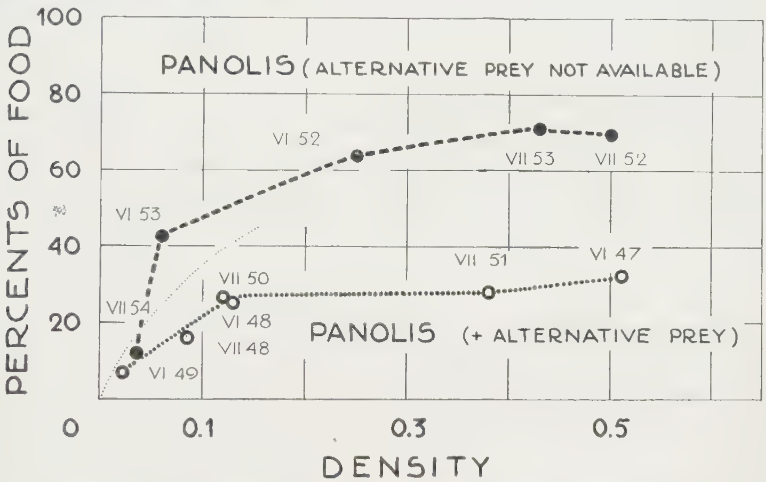


Fig. 14. Percentage in the food and density for *Panolis*. Further explanation of symbols as in fig. 13. See text for the influence of alternative prey.

Here, the data have been split up in two series. One refers to those periods in which large *Panolis* caterpillars occurred together with an appreciable population of other species with a high or moderate risk index (*Lymantria*, *Acantholyda*, or *Diprion* cocoons). The other series comprises those periods in which only prey with a low risk index were found beside *Panolis*. In each series we see a very clear influence of density on percentage in the food. However, there also is a marked difference between the two series, the percentages in the former being much lower than those in the latter. Consequently, if we pooled all data, hardly any effect of density on percentage in the food would be visible.

In this way only those sets of observations have been brought together for each species, throughout this paper, of which it can be

accepted that they have been made under comparable conditions of food availability. Nevertheless the data should be handled with great care. Whenever a certain percentage in the food is lower than one would expect on the basis of the probability of encounter hypothesis, this hypothesis should not be rejected until the possibility has been checked that low relative density of the species concerned is the cause of the low percentage. We shall return to this point on p. 329.

2. SEASONAL FLUCTUATIONS

The food available increases greatly in the course of spring and summer. In most years a peak is reached between June 20th and July 10th. Consequently, the percentage in the food formed by species which occur both early and late in the nestling season can be expected to decrease in the course of time, because, unless the number of individuals of such a species increases proportionally, its relative density will fall. To check this hypothesis, the percentages in the food have been plotted against the accompanying values of density for each species, the first and the second half of the season being treated separately (before and after the appearance of large *Panolis* caterpillars, respectively, figs. 12 and 13).

Four types of prey occur both before and after the appearance of large *Panolis* caterpillars, *viz. Bupalus piniarius*, *Cacoecia piceana*, spiders, and *Diprion sertifer*. At comparable density levels, *Bupalus* moths constituted a much smaller percentage of the food in June '52 than in May of either '52 or '54. The figures for *Cacoecia* suggest a similar state of affairs, but they are not conclusive. It is worth pointing out, however, that in 1952 the appearance of *Panolis* was accompanied by a sudden drop in the *Cacoecia* percentage which could not be accounted for by a sudden decrease in absolute density of the latter (*cf.* table II). Hence, the increase in general food density must be its cause. Spiders in several years formed decreasing percentages in the food from the beginning of May to the end of June (fig. 8). Although we have no reliable measurements of their density, we got the impression that their numbers in the twig samples rise from May to July. This would imply that their risk decreases markedly, unless the increase in their density is insufficient to keep their relative density at the same level. Finally, in *Diprion sertifer*, no definite conclusion can yet be reached, as the density range of the cases observed was much narrower in June than in May, making comparison between the two sets fruitless[1].

On the whole, these facts may suggest that the specific season of occurrence may have an important influence on the toll levied of a species. At least during May, June and July, earliness seems to be correlated with higher predation pressure.

Two factors, apart from the percentage of the food contributed by a given prey species will determine the total number of losses inflicted per day by the birds on this species at a given density. These factors are the number of birds, and the number of prey each of them eats per day. Now in our case, the bird population is doubled between May 15th and June 15th, whereas the feeding frequency drops by about half in that period. Hence, the net effect of the two factors will be roughly constant,

[1] In *Diprion sertifer*, the comparable data are:

Early		Late	
Density	% in food	Density	% in food
0.06	0.25	0.09	0.00
0.09	0.00	0.17	0.00
		0.22	0.05

so that the losses suffered by a prey species will be smallest when its percentage in the food is lowest. This means that late individuals of our insect species, occurring at the same time as large *Panolis* caterpillars, have a better chance of survival than individuals which develop early in the season. It therefore seems a sound inference that natural selection will tend to synchronise the cycles of prey insects with that of *Panolis*. This may be at least part of the explanation of the occurrence of the first of the two peaks of food density (p. 282)[1].

On the other hand, it is reasonable to expect that other selective agents will also influence seasonal periodicity. These apparently predominate in species which have their active stages in periods outside the general peaks. Moreover, these factors may determine that the insects are synchronised in June–July, and not in other months. Climate and the state of food plants may play a role here.

HEINROTH (1924–1926) has suggested that the breeding seasons of palaearctic birds are timed in such a way that most young fledge at the time of the summer peak of food density. The evidence for this view has been strengthened considerably by LACK (1950). The events in our pinewoods certainly support this theory. The period of food abundance, inaugurated by the appearance of large *Panolis*, begins about the fledging date of normal first broods[2]. Thus the parents of these broods rear their nestlings in a period when food is not very plentiful, but conditions improve rather quickly once the young have fledged.

As LACK points out, this coincidence most probably will be favoured by natural selection. Hence, there seems to be a mutual selective influence, the birds tending to synchronise the insect cycles, and the synchronised insects tending to time the breeding season of the birds, the whole complex being ultimately based on other factors, determining the most favourable season for the insects.

Viewed from another angle, the decrease in predation on *Bupalus* and other species in the course of the season shows that the birds have considerable food reserves in their habitat during June and July, which they normally exploit only very little. Before the appearance of *Panolis*, *Bupalus* and *Cacoecia* may provide a large proportion of the food, but thereafter they are taken in much smaller numbers. Yet they are present in the environment up till July. Hence, if in a certain year *Panolis* and the other species of the June peak are scarce, the birds are able to feed on *Bupalus*, *Cacoecia*, and some similar species. We have already seen that pine seeds, spiders and the adults of *Hepialus hecta* L. form a similar latent supply of food during the summer peak of caterpillar density.

XI. THE ROLE OF SPECIFIC CHARACTERS OF PREY IN THE BIRDS' HUNTING BEHAVIOUR

In spite of their conspicuousness, *Diprion pini* larvae are rarely brought to the nest. Yet the birds must often discover them in the wood.

[1] The second peak is caused for the greater part by bivoltine species, such as *Larentia obeliscata*, *Acantholyda*, and some *Diprion* species which all have their first generations in spring and early summer. Some univoltine species, *e.g.*, *Bupalus*, *Atolmis* and *Dendrolimus* also have a peak of larval biomass in autumn.

[2] The fledging dates of the first broods studied by us are often considerably later, owing to the fact that shortly before they fledged we replaced the parent birds' own chicks by another much younger brood taken from elsewhere. This provided a valuable extension of the period of observations, since the replacement does not affect the parental behaviour of the old birds, who in this way can be made to raise as many as three broods in succession.

Evidently they do not take them, or at least they do not use them as food for the nestlings. N. Prop (in this volume) observed the behaviour of Great Tits at feeding tables on which a variety of prey species were offered. Under these circumstances, the *Diprion* larvae were rarely touched by the birds at all, whereas *Panolis*, *Acantholyda*, *Bupalus* and similar species were taken at first sight. These experiments were made with wild birds in pinewood, and for these birds visual characters evidently were sufficient to distinguish between different species.

It is worth finding out how the birds use these characters. The hunting process can be thought to have two components, *viz.* 1. the search and 2. what happens after a prey has been discovered. Now one could suppose that searching is unspecific, that is to say that prey is discovered by means of characters common to all or most species. The specific characters would then be used in the second phase, and would serve for a check of the quality of the prey after it has been found. However, this simple assumption is not confirmed by the facts, and it will be shown that specific prey characters most probably are very important even in the search for a prey.

Already in the first years of this investigation we got the impression that there is often a time lag between the changes in the fauna and those in the birds' food. When a new species appears in the environment, its representation in the food goes up considerably later than its density. It will now be shown that this fact leads to a more complicated view of the use of specific characters in gathering prey.

1. THE APPEARANCE OF NEW SPECIES OF PREY

Fig. 15 summarises food and density figures at the beginning of the *Acantholyda* season in 1948 and 1950. Clearly, consumption increased disproportionately (as compared with the change in density) between the beginning and the later part of each period of observations. Moreover, the differences between breeding pairs are remarkable.

As far as concerns the first point, it should be stressed that the density figures refer to all individuals more than 10 mm long. This is about the smallest *Acantholyda* taken. Now within this category, size might have increased with time during the observations. This would almost certainly have entailed a rise in risk (p. 294). In that case, the density figures alone would give a misleading impression of availability. However, the few data on size which we collected, and which are added at the bottom of fig. 16, seem in contradiction with this view. In 1948, the average size of specimens in the twig samples was almost the same in both parts of the period. In 1950, although there was an increase, size was already far above the lower threshold of acceptab-

ility during the earlier part of the observations. Yet three out of six birds still had very low percentages of *Acantholyda* in the food on 5, 6 and 7 June. These percentages rose steeply on the 8th. This sudden change suggests that some factor other than density or size was involved, though it must be admitted that the average sizes were determined in very small samples. Hence, the observations do not permit a definite conclusion, but only suggest that size is not responsible for the low rate of predation during the beginning of the *Acantholyda* season.

In any case, one other obvious explanation, namely that at first the birds were not hunting on the places where *Acantholyda* lives, can be discounted. In both years they were feeding in the crowns from the start.

As a third possible explanation, it might be supposed that the birds, although hunting in the crowns, must first learn to catch *Acantholyda*, and that this learning process took some time. At first sight, this view is supported by the fact that the *Acantholyda* percentage rose at different dates in the different pairs. However, in 1950 the partners of each pair behaved in a very similar manner. Now if a learning process is

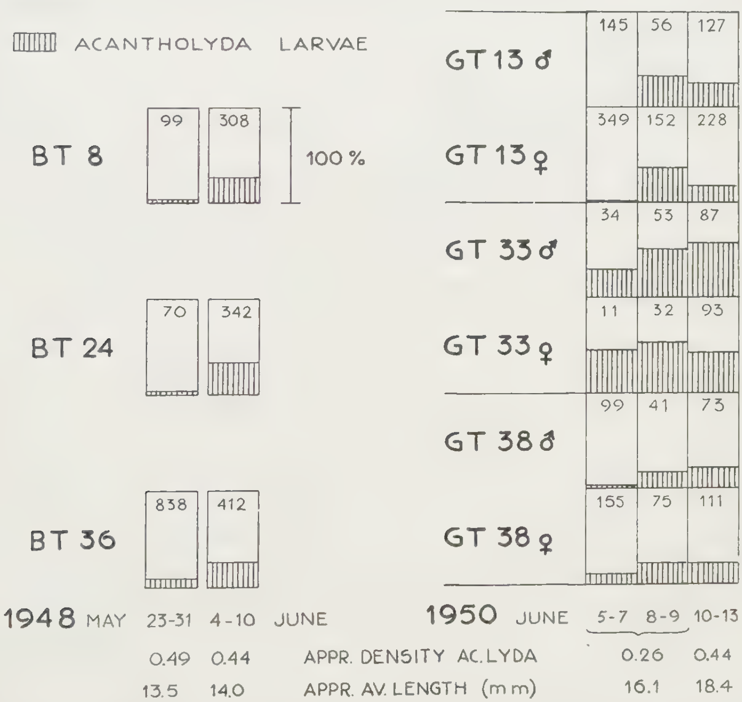


Fig. 15. Percentage in the food and density for *Acantholyda* in 1948 and 1950. The figures in the columns denote the total number of prey identified in each period.

involved, one might expect this to take place at different times in the partners of one pair, and this in fact has been found in some cases (p. 314). Therefore, the differences among pairs may be due to differences in the prey populations of separate tit territories, *e.g.*, *Acantholyda* might have developed earlier in the territory of GT 33 than at other places. Clearly the observations do not suffice for a choice to be made between the alternatives. We need more information on the sizes of prey, and their densities and times of appearance in the territories of the various pairs of birds.

To these problems B. A. Bennema has devoted a special investigation in the summer of 1952. For technical reasons, *Panolis* was studied instead of *Acantholyda*. The density of different instars was measured by the faecal pellet method. In each tit territory under observation 15 frass collectors were erected on June 4th, and emptied on the 9th and 17th. The weather was favourable during this time. There was some rain, but it was evenly distributed over both intervals. No great changes in temperature occurred.

In the faecal pellet counts, three size-classes were distinguished, corresponding to the third, fourth, and fifth instars, respectively. Of these, the third is very rarely taken by tits and will be disregarded in the following discussion. The fourth (18–25 mm long) runs a moderate risk, whereas the fifth instar (25–40 mm) is a highly valued prey (p. 294). In the preceding sections, we have compared density and consumption for all *Panolis* more than 20 mm long. Here, however, the fourth instar is excluded, and predation figures have been correlated with the denstiy of the fifth instar only. Thus, the disturbing influence of size has been eliminated as much as possible.

In the critical period, five Great Tit pairs (4, 22, 30, 13 and 36) were available. Food observations were made each morning from 7 to 10 a.m. These data we used to calculate the average percentage of *Panolis* in each day's food, and also to examine the sequence of appearance of *Panolis* in the food of the various tits.

Two of the pairs mentioned (13 and 36) had already been studied for three weeks before the beginning of this series of observations (their nestlings had twice been changed for younger ones). Feeding a great deal on *Cacoecia* larvae and pupae, they had spent most of their time in the crowns. During the last days of May and early in June, however, three out of these four birds (♂ 36, ♀ 36 and ♀ 13) had caught a great number of emerging *Bupalus* moths from the ground in the morning hours. The remaining part of the day they still fed in the crowns. This ground-feeding lowered the percentage of tree-living prey in the food. For this reason, the figures of pair 36 were not used in the calculation of the daily average percentage of *Panolis* in the food (see also p. 313).

The three remaining pairs (22, 30 and 3) took their food from the crowns throughout the observation period. Correspondingly, they had a high proportion of *Cacoecia* in their food during the first days after the appearance of *Panolis*. It is most

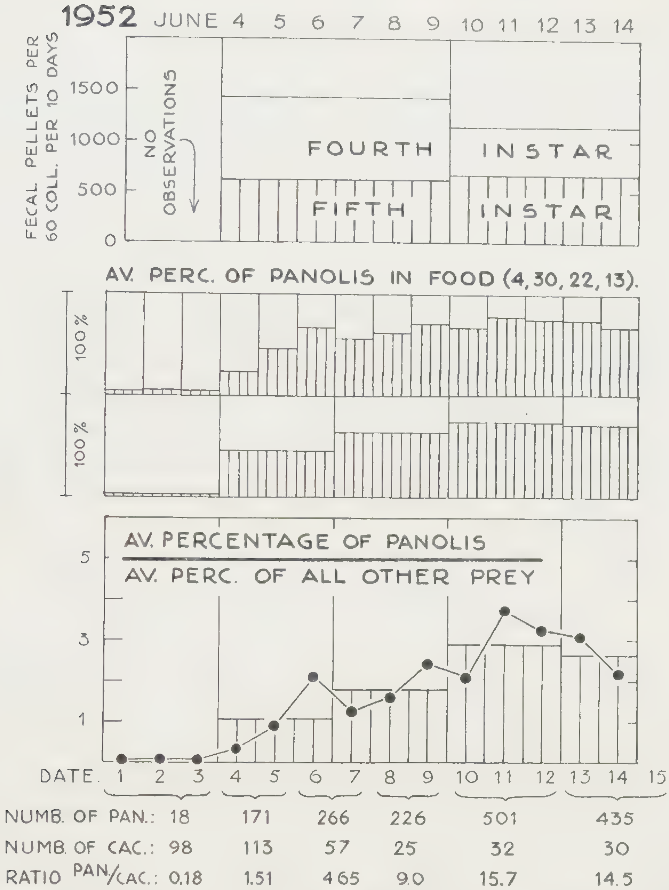


Fig. 16. Time course of the occurrence of *Panolis* in the environment and in the tits food. See text.

unlikely that in these birds a low rate of predation on *Panolis* during these days would be due to their feeding away from the crowns.

A summary of the observations is given in fig. 16. The density of fourth instar *Panolis* was found to decrease, but, very fortunately, the average density of the fifth instar, as derived from frass production, was about the same in the first and the second part of the period (fig. 16, top). In contrast to density, however, the average daily percentage of the fifth instar in the food increased gradually (fig 16, middle).

Now no other species, *e.g.* no *Acantholyda*, were entering the prey fauna, and the losses of *Cacoecia* during the observation period were relatively slight (see below). Hence, if the risk index of fifth instar *Panolis* were as constant as its density, its percentage in the total

food could be expected to remain nearly unchanged during the observations.

As already stated, however, the actual figures show a very different picture. There is a marked rise in predation. Only after about the 10th of June does the percentage of *Panolis* in the food remain constant. Evidently the risk index of *Panolis* must have increased (fig. 16, bottom: assuming the quotient of the densities of *Panolis* and all other food to be constant, the quantity plotted in this graph is a direct measure for R_{Pan} / R_o, as can be seen from the calculations in ch. XII).

A certain measure of uncertainty, however, is introduced into the argument by the fact that the birds hunted part of the time on the ground. Therefore, the increase in risk of *Panolis* can be demonstrated even better by comparing the numbers of *Panolis* and those of *Cacoecia* in the food, since both are affected by ground feeding in exactly the same way. The ratio of these numbers changed quickly between the 4th and 9th of June, and then remained the same. Average values are:

	Cacoecia/*Panolis*
4–9 June	0,293
10–15 June	0,066

Now the mortality of *Cacoecia* was less than 25 % per week in the period referred to. On the basis of the probability of encounter hypothesis (p. 293), taking this value and a 10 % increase in *Panolis* density into account, we would expect the ratio to amount to 0.213 instead of 0.066 between the 10th and 15th of June. Again, an increase in the risk index of *Panolis* seems indicated.

Thus far, the observations in 1952 confirm the suggestion derived from the data on *Acantholyda*. However, we must still consider the possibility that the increase in size of the fifth instar *Panolis* larvae during the observation period was responsible for their increase in risk. Although a young fifth instar larva is already a very attractive prey, its risk index can indeed be expected to increase somewhat with size. The increase in average size, however, will have been much smaller than growth rate alone suggests. The toll levied by birds on *Panolis* caterpillars is very high. For this reason, old individuals are always scarce in the population. Now in the second part of the period of observations, the rate of predation was much higher than in the first part. This factor, together with the entrance of young individuals into the population, will have reduced the increase in average size of the fifth instar larvae. We therefore can assume that the rise in risk was only to a small extent due to the increase in average size. This view is supported by the fact that *Panolis* larvae more than 30 mm long

are rare in the food of tits, most of those brought being between 25 and 30 mm.

This consideration, which is supported by additional evidence given below, leads to the explanation that the main cause of the increase in risk lies in the birds and not in the caterpillars. Evidently the tits change their behaviour towards *Panolis*.

So far as concerns Great Tit pairs 4, 22, and 30 (fig. 17), this change might be a consequence of the age of their young, which was 3–5 days on the 2nd of June. The desirability of small prey might have urged them to look for other species. This, however, is improbable. In the first place, pairs 13 and 36 show the same picture, although on June 2nd their nestlings were 13 days old. Secondly, the preference for smaller prey is strongest during the first two days after hatching, which were excluded from our observations on pairs 4, 22 and 30. Moreover, the rise in the *Panolis* percentage in the food of these pairs is much greater than that found in cases where *Panolis* had been present in the environment for some time before the rearing of the young began. In the latter cases, averaging all second broods which we have observed from the beginning of the first till the end of the third five days' period, the percentage of *Panolis* in the food rose from 28.4 % in period I to 33.2 % in period II and 33.9 % in period III.

In conclusion, we are convinced that the increase in predation on *Panolis* shown by pairs 4, 22 and 30 was not an effect of the age of the young. We consider it a consequence of the appearance of the species in the environment. Evidently the tit population reacts to this event by a delayed increase in its pressure on the species.

In pairs 4, 22, 30 and in ♂ 13, the increase in the percentage of *Panolis* was offset by a decrease in that of *Cacoecia* and spiders. In ♀ 13, it was also correlated with a strong decrease in the percentage of *Bupalus* moths, which occurred in spite of constant average density of emerging moths on the ground throughout the observation period. Hence, the increase in *Panolis* percentage in this case partly originated from a shift in feeding places, which, however, was not observed in the other pairs. The values plotted in fig. 16 refer to pairs which foraged almost exclusively in the crowns.

When the food of individual tits is considered (fig. 17), it is in the first place remarkable that the increase in the percentage of *Panolis* was often very sudden. This is an additional reason for believing that, apart from density and size (which would both cause a more gradual increase), some other factor plays an important part.

Further, there were marked differences of as much as several days among the dates at which *Panolis* appeared in the food, the sequence being pair 30, 36 ♂, pair 13 and pair 22, pair 4, 36 ♀. We must now consider the sequence of appearance of *Panolis* in the different territories. It can be assumed that a high ratio of fifth-instar to fourth-

instar faecal pellets indicates an early appearance and a low ratio a late one. The detailed records available show that territory 4 was earliest of all and that it was followed by 30, 13, 22 and 36, which was latest. Consequently, the sequence in the food was clearly different from that in the fauna. In the hunting area of pair 4, *Panolis* appeared early, but in their food it was late, even though this pair had older young than pairs 22 and 30. This is the more remarkable as the density of *Panolis* was higher in this territory than in any other. Similarly, *Panolis* was late in territory 36, but ♂ 36, although hunting a great deal on the ground, was rather early; ♀ 36, however, behaved in a different way and was later. This proves once again that individual characteristics play a part[1]. Likewise, ♂ and ♀ 22, although they started to feed *Panolis* on the same day, show a quite different pattern. In the ♂, the *Panolis* percentage showed a slow increase between the 4th and 11th of June, whereas in the ♀ it became steady as early as the 5th. This difference occurred in spite of the fact that the birds were confronted with the same prey population. Finally, there is also a difference between ♂13 and ♀13, but it is uncertain whether this is real, some of the food samples being rather small.

Despite these differences, the partners of each pair (except 36) were remarkably similar in the dates at which they started to bring *Panolis* in appreciable numbers. As stated on p. 310, the same was found in *Acantholyda*. Contrary to expectation, however, the density figures for *Panolis* show that this similarity was not caused by the availability of that species in the respective territories. Hence, another influence must be assumed.

Summing up, we have to deal with three points. After the development of a rather dense population of *Panolis* caterpillars in the territory of a pair of Great Tits, predation pressure remained very low at first and then increased rather quickly to a high level. The date of this increase was not the same for all pairs, and there was no fixed relation between this date and the condition of *Panolis* in the territory

[1] ♀ 36 was late (6 June); at first sight, one might suppose that this was a result of much ground feeding, which obviously will lower the percentage of *Panolis*. However, the ratio $\frac{Cacoecia}{Panolis}$ which is independent of ground feeding, also changed suddenly on June 6th:

Dates	1, 2, 3	4, 5	6, 7	8, 9	10, 11, 12	13, 14, 15 June
♀ 36 Number of *Panolis*	2	2	24	10	no	observations
Cacoecia	16	10	6	3		
Ratio *C/P*	8.0	5.0	0.25	0.30		
Pairs 4, 22, 30, 13 Ratio *C/P*	5.6	0.66	0.21	0.11	0.06	0.07

in question. Finally, the members of a pair generally were synchronised in their reactions towards *Panolis*, again more or less irrespective of density.

The question arises whether this is the usual situation when a new species of prey appears in the environment. In fact, the observations on *Acantholyda* point in the same direction, but the accompanying

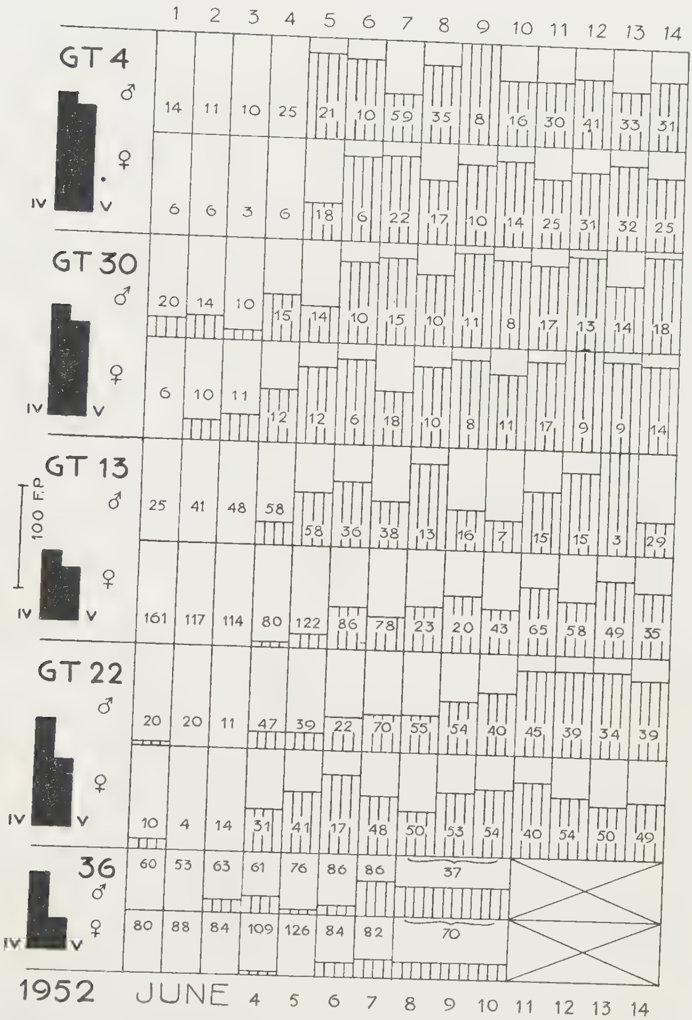


Fig. 17. The time course of *Panolis* percentages in the food of individual Great Tits in 1952. The figures in the columns denote the number of prey identified on each day. The height of the columns represents 100% of the daily food, except in pair 36, where it represents 50%. The black columns at the left show the number of 4th and 5th instar *Panolis* faecal pellets collected in each territory on June 9th.

measurements of density and size are fragmentary. In *Bupalus* moths, however, J. and L. Mook were able to establish the same three points as made here for *Panolis* (for details, see Mook and others, in this volume). This is especially important because differences in size are completely excluded here as a factor which may increase risk in the course of the season. For other species we have fragmentary evidence which nevertheless suggests that these follow the same pattern.

We must now try to explain these phenomena. In principle, the low risk of a new species in the first period after its appearance could arise in two ways. Either such prey was found, but not accepted by the birds, or it was not found at all. In the case of *Panolis*, the former assumption is very improbable. The feeding experiments always showed that big larvae of this species are very attractive prey. Moreover, Miss C. S. Duintjer made the following experiment which proves that Great Tits very quickly accept prey which they had not seen earlier. Two Great Tit pairs, which regularly took mealworms from feeding tables, were confronted with a very unusual kind of prey: mealworms painted bright green with a conspicuous white cross band on each segment. These were offered together with an equal number of ordinary mealworms. The results of this experiment show no preference whatever. The tits had not the slightest aversion to this very abnormally coloured prey. Hence, it is very difficult to believe that for some days *Panolis* larvae were found but not accepted by the birds. It follows that they must have been overlooked during these first days. Accordingly, our results are to be explained by assuming 1. that specific characters are used in the search for prey (and not only in testing prey once it has been discovered), 2. that these specific characters are assimilated in a kind of learning process, by means of which the birds adopts a "*specific searching image*", and 3. that one member of a pair adopts the searching images of its mate when seeing the prey collected by the latter. In other words, we believe that a given species of prey can only be collected effectively when the birds use its specific characters. Otherwise they will catch only a few individuals which they find by chance. It may be supposed that such a specific searching image is assimilated when the birds have had a number of chance contacts with the species in question. Individual characteristics and previous specialisation would account for the differences between pairs which one after another adopt the searching image of a newly appearing species, and, by applying it, greatly increase their pressure on the latter. As regards the taking over of the mate's searching image, it should be added that the pair often hunt together, and also often seek each other's company in the neighbourhood of the nest.

Although this explanation is not in contradiction with any known facts, it is difficult to give a more rigid proof by means of field observations. The principle of specific searching images, however, has been demonstrated in a more strict way by the work of De Ruiter (1952, 1953). This author experimented on the protective value of concealing patterns in caterpillars against birds, mainly Jays *(Garrulus glandarius)*. These had to find caterpillars hidden in vegetation or other appropriate cover. De Ruiter was able to show that the effect of their searching increased very much as soon as they had happened to find a first specimen of the caterpillar species tested.

In this connection, it may also be pointed out that the efficiency of human searching is greatly increased when the details of the object sought are known. This is especially true in complicated surroundings. Here, too, it can be said that the person seeking must have a specific searching image in mind.

The explanation presented here implies that the composition of the food taken cannot be predicted on the basis of a constant risk index. The rate of consumption depends greatly on whether a specific searching image is adopted or not. Therefore, the factors which govern this process are very important for an understanding of the composition of the food. These factors will be considered in the following sections.

Finally the facts mentioned in this section have an important implication for our calculations. When studying the risk of different species it is necessary to exclude those periods in which the species in question is just entering the prey fauna. This condition has been fulfilled throughout the present paper.

2. SHORT-TERM FLUCTUATIONS IN THE COMPOSITION OF THE FOOD

In the observation hides, one often gets the impression that the prey is brought in small batches of the same species and not in a random sequence. In a number of cases, this impression is confirmed by a statistical analysis.

The phenomenon may have various causes. As mentioned on p. 283 the food flights of tits are usually directed to the same part of the wood for a short while, after which the birds shift to another place, *etc.* Likewise, they tend to make a number of successive visits to the same stratum in the wood. If a certain species of prey is not distributed at random in the habitat, this periodicity of the feeding flights may lead to periodicity in the food.

Some examples of this kind were found in the course of our observations, but we also recorded feeding in batches when all species involved lived in the same layer of the wood. This result suggests a

temporary preference for a certain searching image. We shall deal with this question at a later occasion.

3. INDIVIDUAL DIFFERENCES IN FOOD TAKEN

When several broods of the same age were studied at the same time, we generally found individual differences in the composition of the food. Figs. 18–20 represent a number of cases in which these differences

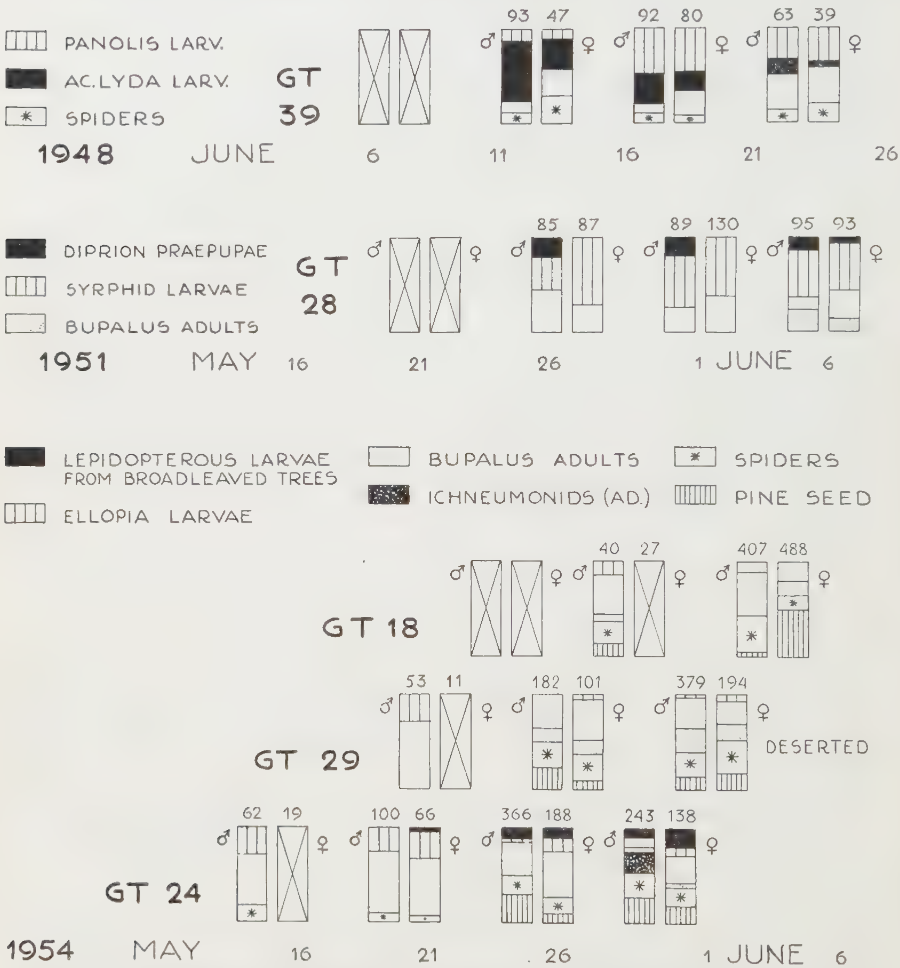


Fig. 18. Composition of the food of individual tits, calculated over 5 day periods, beginning at hatching. The numbers denote the total number of prey identified in each period. White areas in the columns: proportion of food made up of other types of prey than those specified. The height of the column represents 100% of the food in all cases.

were great. It is obvious that several causes may lead to this variability, for example:

1. Differences in the availability of prey

Pair Great Tit 24 (1954) had high percentages of caterpillars from broad-leaved trees in the food. Pair 29 and pair 18, which were studied about the same time, had a very low percentage. Nest 24 was situated at a corner of the pinewood. An open wood of birches is found 50 meters away. The other pairs had only a few broad-leaved trees in their neighbourhood (fig. 18).

2. Conditioning to feeding in different layers of the wood

♂ Great Tit 28 (1951) brought small numbers of *Diprion pini* praepupae throughout the nestling period. In the food brought by his mate, these were recorded only during the last 5-day period. The praepupae were collected on the ground. We observed that the ♀ did much less ground feeding than the ♂. As soon as she came on the ground, she also brought *Diprion*. This is demonstrated in the graphs by the simultaneous appearance of *Diprion* and emerging *Bupalus* moths in her diet (fig. 19).

3. Differences in feeding technique

In 1946, we studied a pair of Great Tits in an oak copse. Both birds collected their food in the crowns. The composition, however, was rather different. In the food of the ♀, 43% consisted of the grasshopper *Meconema thalassina* De Geer, whereas the ♂ had

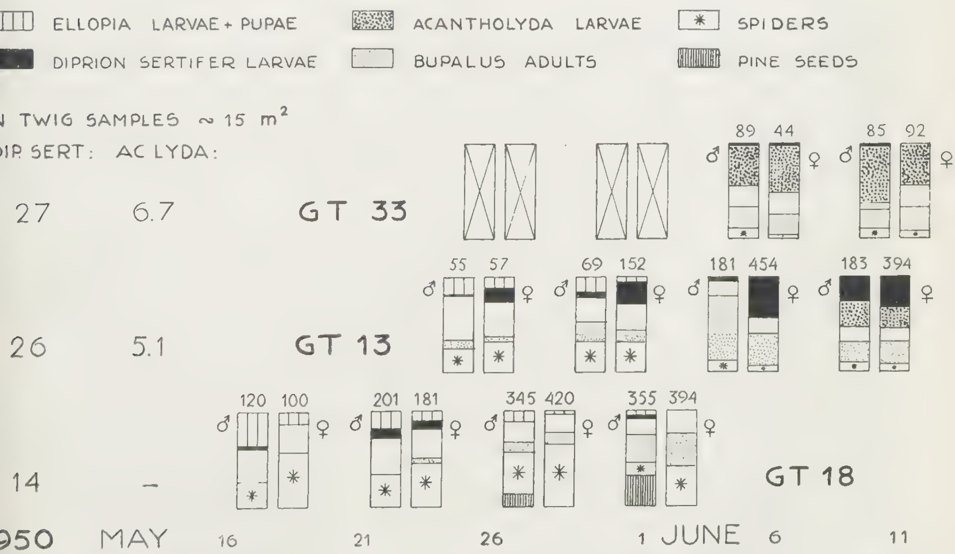


Fig. 19. Composition of the food of individual tits, explanation see fig. 18. The density figures, which always refer to larvae over 10 mm long, were determined during the third and fourth 5 day periods of each brood.

only 7% of this species in his diet. The grasshoppers were sitting on the undersides of the leaves. Now the ♀ had a highly specialised hunting technique. Most of the time she was seen jumping through the shrubs from bottom to top, the head pointing upwards. In this way she inspected the undersides of the leaves. The ♂ did not show this behaviour but searched at all kinds of places, the uppersides of the leaves, the twigs and the branches included.

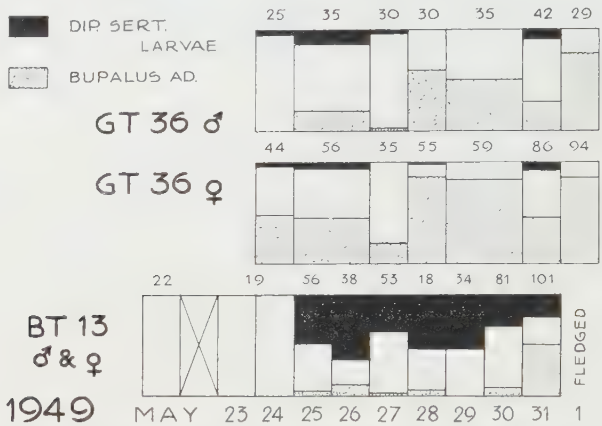


Fig. 20. Percentages of *Diprion sertifer* and *Bupalus* in the food of two pairs of tits in 1949. The height of each column represents 100% of the food. White: other food.

Although these three factors explain the examples cited above, individual differences in food are often due to other influences. To show this, a number of cases will be discussed in which it can be proved that factors (1) and (2) are not responsible for the differences found. In all of them, factor (3) can be excluded beforehand, as they refer to kinds of prey which are readily accessible and which have no special escape reactions.

a. Great Tit ♂ 24 (1954), (fig. 18) brought a high percentage of adult Ichneumonids[1] during the last 5-day interval of the nestling period. The wasps were very abundant in all parts of the wood, where they swarmed over the moss layer up to a density of 1 per 2 sq. m. in each territory. The percentages for *Bupalus* and pineseeds show that all birds did much ground feeding. This was confirmed by direct observation. Nevertheless only ♂ 24 collected Ichneumonids.
b. In the same set of data, pineseed is the most important food item for ♀ 18. Like its mate, this bird collected most of its food on the ground. The ratio *Bupalus*/pineseed, however, was quite different in the food of the partners.
c. ♂ and ♀ 39 (1948) both fed in the crowns, and consequently were faced with the same population of prey. Nevertheless the percentage of *Acantholyda* was much higher in the ♂ than in the ♀. This difference persisted throughout the nestling period.
d. ♂ and ♀ 4 (1953) both collected most of their prey in the crowns of the pines. The food record was the following:

[1] According to Mr. P. Kuyten very probably *Cratichneumon nigritarius* Grav. ♂♂.

	♂	♀
Total number of prey	90	115
Diprion nemoralis larvae	3.5%	22%
Panolis larvae	63 %	42%

The food of two other pairs during the same period was about the same as that of ♂ 4.
e. Great Tit 18 ♂ (1950), (fig. 19) was the only bird among six individuals which brought an appreciable number of pine seeds. Yet this food was abundant in every territory, and all the birds hunted part of the time on the ground.

Several more examples of the same kind could be added. In all of them individual differences in the food were due neither to differences in the availability of prey species, nor to a preference for different layers in the wood, nor to differences in feeding technique. Evidently some other factor was at work here, which must have been internal and individual.

These cases suggest that some of the birds used searching images that other members of the population had not accepted. Yet it may be questioned whether the examples prove the existence of searching images. The possibility of alternative explanations must be examined. The most reasonable alternative is that the birds all found the same prey, but had individually different tastes and therefore brought different collections of prey to the nests. It does not seem possible to exclude this alternative in the examples just mentioned.

A few other cases, however, can only be explained in terms of searching images. We mention here the most suggestive example. Great Tit 36 and Blue Tit 13 (1949) showed a remarkable difference in diet (fig. 20). The main prey were larvae of *Diprion sertifer* and adults of *Bupalus*. Both were very abundant in the wood. Although other Great Tits were found to take many *sertifer*, ♂ and ♀ 36 specialised on *Bupalus* and collected great numbers of these in the crowns[1]. The Blue Tits of pair 13, on the other hand, fed mainly on *Diprion sertifer* (which they obviously also caught in the crowns), and almost neglected the moth. This is not a specific difference, for we observed several Blue Tits, which brought great numbers of *Bupalus* to their nests. Moreover, on the last day of the observations, the birds of pair 13 suddenly switched over and brought a high proportion of *Bupalus*. This late start is the more remarkable as choice-experiments by N. Prop and Miss Duintjer showed that *Bupalus* adults are very attractive to tits, whereas *Diprion sertifer* larvae are decidedly unattractive. It is very improbable therefore that these Blue Tits did find but rejected *Bupalus*

[1] As already mentioned, this is unusual. As a rule, Great Tits take *Bupalus* from the ground. In cold weather, however, when the moths are slow, they may be caught in the crowns. This rule does not apply to the Blue Tit, which catches many *Bupalus* in the crowns in good weather, and rarely hunts on the ground anyway.

between May 25th and 30th. A far more reasonable explanation is that, by chance, they did not adopt the searching image until May 31st.

Although the evidence is not complete, we believe that in the other cases of individual specialisation, too, the individual tits used different searching images. This leads to the question why a certain searching image is adopted by one individual and not by another. We should realise here that the birds generally use a number of different searching images in combination, and that even the food of a specialist shows much overlap with the food of other individuals. The difference affects only one or two species of prey. Hence, it is better to examine first, which factors determine the formation of searching images in general. A detailed discussion of this problem must be postponed till p. 332, but we can anticipate here that palatability, size, conspicuousness, density and the availability of other kinds of food are the most important influences. Although the requirements in these respects will be about the same in all Great Tits, as is shown by the general similarity in food, some individual variation can be expected. Thus the fact, that in 1953 only Great Tit 4 ♀ (see above) specialised on the unpalatable larva of *Diprion nemoralis* might be the consequence of an individually different threshold. In other words, it might be supposed that the other birds would have followed if the conditions had been somewhat less favourable.

In other cases, however, an influence of chance seems more probable. The above-mentioned Blue Tits 13 (1949) are an example. Another case was found in Great Tits. GT 13 and 33(1950), (fig. 19), had about the same densities of *Diprion sertifer* and *Acantholyda nemoralis* in their territories. ♀ 13 was a *Diprion* specialist from the start of the nestling period, whereas the ♂ took low percentages at first and suddenly increased them in the fourth 5-day interval. Pair 33 on the other hand took only very small numbers of *Diprion sertifer* and specialised on

TABLE IX

Food of Great Tits 13 (1950)

	5 day intervals I	II	III	IV
♂ 13				
Number of *Diprion sertifer*	1	3	7	44
Number of other crown living species[1]	14	19	15	5
Ratio *sertifer*/others	0.07	0.16	0.47	8.8
♀ 13				
Number of *Diprion sertifer*	7	35	193	134
Number of other crown living species[1]	11	13	17	2
Ratio *sertifer*/others	0.64	2.7	11.3	67

[1] *Acantholyda* has been excluded because this species appeared in the course of the observation period.

Acantholyda. The latter prey appeared in the food of GT 13 only in the last 5-day interval.

The rise of the *sertifer* percentage in the food of ♂ 13 was accompanied by a fall in the *Bupalus* percentage. One might suppose that this ♂ only changed from ground feeding to twig feeding. In fact, however, there was also a clear specialisation on *Diprion*. This can be shown by calculating the ratio between the number of *D. sertifer* and that of other species from the crowns (*Ellopia* larvae and pupae, *Evetria* larvae, *Cacoecia* larvae and *Dendrolimus* larvae taken together, see table IX). In the environment this ratio must have increased very slowly since these other species decreased slowly in numbers. In the food, however, the increase was very strong, which can only be due to specialisation on *Diprion*.

Obviously ♂ 13 specialised on *D. sertifer* much later than his mate. By that time he could also have specialised on *Acantholyda* as did ♂ and ♀ 33. Most probably, the accidental circumstance that his mate was a *sertifer* specialist made him the same.

Summarizing this section, we may conclude that individual differences in food are caused by both external and internal factors. We believe that among the latter searching images are important. In at least one case, no other explanation seems possible. It appears that under the same conditions different individuals do not adopt the same searching images. In some cases this may be caused by differences in food preference, in others it is doubtless due to chance.

XII. THE RELATION BETWEEN THE DENSITY OF DIFFERENT PREY SPECIES AND THE COMPOSITION OF THE TIT'S FOOD

1. EXPECTATION BASED ON THE PROBABILITY OF ENCOUNTER HYPOTHESIS

We shall now examine more closely the influence of the density (both absolute and relative) of a prey species on its percentage contribution to the food.

All available data are combined in fig. 21, in which for various prey species the percentages in the food have been plotted against the respective absolute densities, both on a logarithmic scale. These graphs are to be compared with figs. 12–14, which present the same data on a linear scale, the advantage of the logarithmic scale being that at low densities the picture is more clear.

It may be useful first to consider briefly the shape such a graph will take if the probability of encounter hypothesis is correct. We have seen (p. 293) that in that case

$$N_A = R_A \cdot D_A \cdot t$$

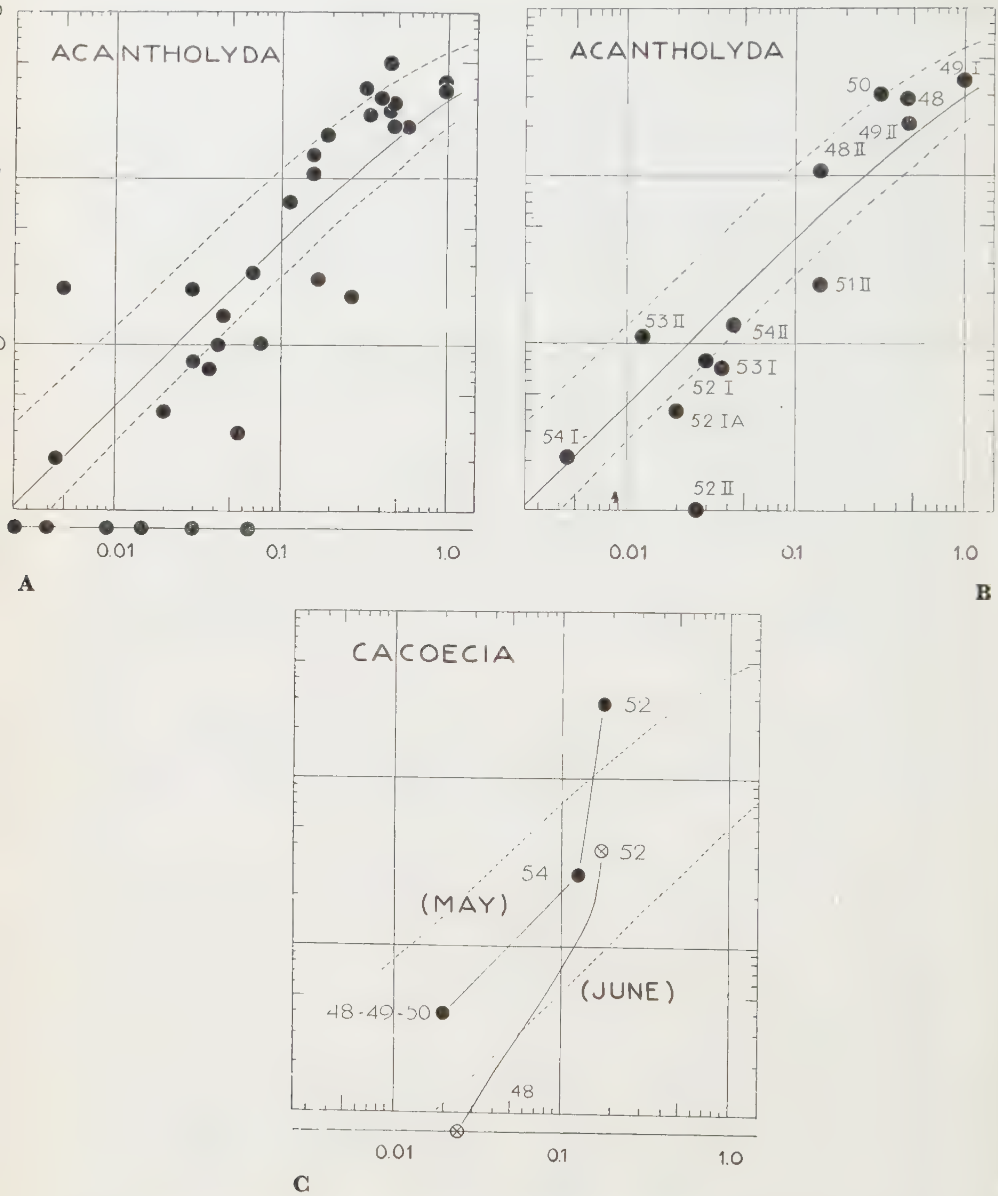
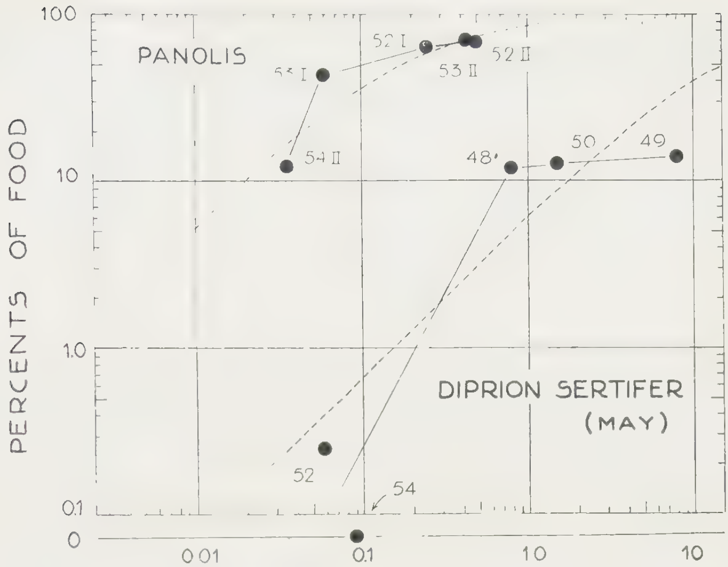


Fig. 21 A–C. The relation between density and percentage in the food for a number of prey species. Broken lines in all diagrams, and full drawn lines in those for *Acantholyda:* expectation curves. A. *Acantholyda*, observations on individual pairs (table x) B. *Acantholyda*, averages for first (I) and second (II) broods in various years (arabic numerals). C. *Cacoecia*, before (May) and after (June) the appearance of *Panolis* and *Bupalus*.

D



E

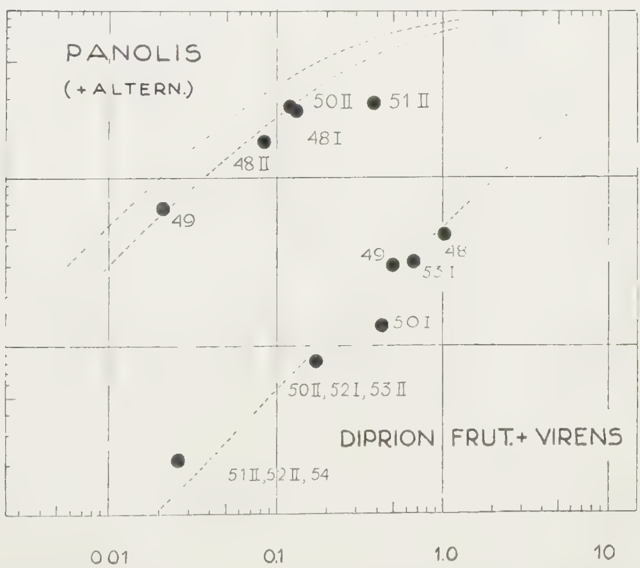


Fig. 21 D–E. Explanation see fig. 21 A–C. D. *Panolis* in the absence of alternative prey, and *Diprion sertifer* before the appearance of *Panolis*. E. *Diprion frutetorum* and *virens* (in the presence of *Panolis*), and *Panolis* (in the presence of alternative prey). In the last mentioned graph, the 1947 observation (mentioned in fig. 14) has been omitted because it is based on 1 Blue and 2 Crested Tit pairs. Note: logarithmic scale on both axes.

so that the percentage P_A of A in the food

$$P_A = \frac{N_A \cdot 100}{N_A + (N_B + N_C + \cdots + N_{M-1} + N_M)}$$

$$= \frac{R_A D_A \cdot 100}{R_A D_A + (R_B D_B + R_C D_C + \cdots\cdots + R_M D_M)}$$

which, for simplicity, may be written as

$$P_A = \frac{R_A D_A \cdot 100}{R_A D_A + R_O D_O}$$

in which D_O denotes the total density of all prey other than A (*i.e.*, the sum of the densities of the individual species) and R_O the average risk of a prey individual belonging to this group (which can be calculated from the risk indexes of the individual species, weighted for the density of the species concerned).

Fig. 22 shows a number of curves representing P_A as a function of D_A, obtained by substituting various different values for $\frac{R_O \cdot D_O}{R_A}$ in

$$P_A = \frac{R_A D_A \cdot 100}{R_A D_A + R_O D_O} = \frac{100}{1 + \frac{R_O D_O}{R_A} \cdot \frac{1}{D_A}}$$

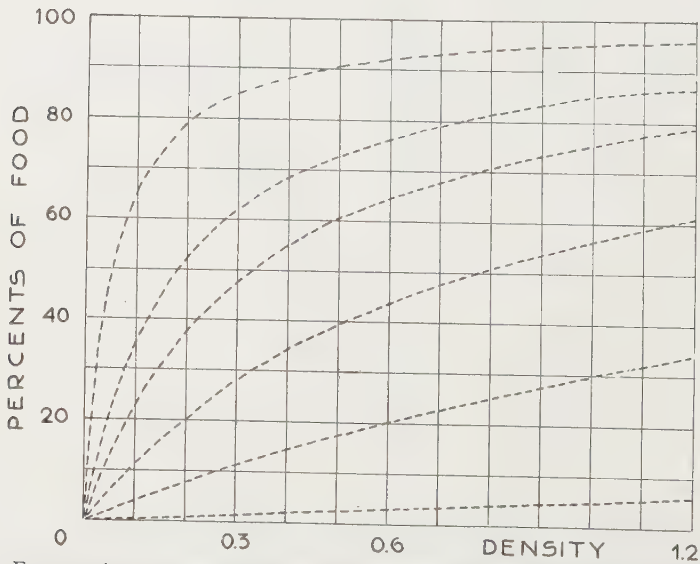


Fig. 22. Expectation curves. From top to bottom, the following values have been used for $\frac{R_O}{R_A} \cdot \frac{1}{D_A}$: $^1/_{20}$, $^1/_5$, $^1/_3$, $^3/_4$, $2^1/_2$ and 20.

We shall refer to such curves as expectation curves. It must be remembered that they are based on the assumption that the risk index is independent of the density of the species concerned. Fig. 22 illustrates the obvious fact that the percentage of species A in the food varies both with R_A and with $R_O D_O$.

In fig. 21 we have added to each set of data plotted one or more expectation curves, calculated for values of $R_O D_O / R_A$ suggested by the respective observed food percentages and densities of A[1].

2. OBSERVED RELATIONS

Among the species studied, only the green *Diprion* larvae (*virens* and *frutetorum*, combined) can be said to follow the accompanying curve of expectation. The situation in *Panolis*, *Acantholyda*, *Cacoecia* and *Diprion sertifer* can be described as follows. At the lowest densities examined, the percentage in the food is relatively low. At somewhat higher densities it shows a much steeper rise than the expectation curves. Consequently, it reaches a relatively high value at medium densities. Finally, at still higher densities, the slope in the curves of the observed percentages is much less steep than the slope of the expectation curves. Evidently, the relative intensity of predation reaches a maximum at moderate absolute densities of the species concerned. The actual value of this moderate density is different for each species. It is lowest in *Panolis*, intermediate in *Acantholyda* and *Cacoecia*, and highest in *Diprion sertifer*.

This account is in part an extrapolation, for the higher branch of the curve is absent in *Cacoecia*, and the lower branch in *Panolis* (in the presence of alternative prey), owing to the fact that higher densities of *Cacoecia* and very low densities of *Panolis* have not been encountered during the observations. In *Acantholyda*, the picture is nearly complete, although only the beginning of the flattening of the curve at high densities has been observed. In addition, it should be mentioned that the observations on *Panolis* (in the presence of alternative prey) seem to follow the expectation curve over a rather wide range of densities, extending from 0.02 to about 0.13.

Thus the observations form a complicated picture, and the next step must be to examine the possible causes of the relations observed.

Before we take this step, however, we must investigate the statistical significance of the deviations from the expectation curves. Only in the case of *Acantholyda* there is enough material for a quantitative discussion

[1] For example, if $D_A = 0.1$ and $P_A = 30$, $(R_o \cdot D_o)/R_A = (100 - P_A)D_A/P_A$ equals 0.233. P_A can now be calculated for all other values of D_A, *e.g.*, if $D_A = 0.01$, we find $P_A = 4.1$ per cent. of the food.

TABLE X

The relation between risk and density in Acantholyda. C_{Ac} = percentage Acantholyda in the food, D_{Ac} = population density of Acantholyda, K = 1/S. See text

Year	Pair	C_{Ac}	D_{Ac}	K	Year	Pair	C_{Ac}	D_{Ac}	K
1948	BT 36	20.5	0.58	0.44	1952	I	0.8	0.03	0.27
I	BT 8	35.0	0.32	1.64	1952	IA	0.4	0.02	0.20
	BT 24	34.2	0.99	0.52	1952	GT 30	0.0	0.063	0.0
	BT 13	30.4	0.40	1.10	II	GT 14	0.0	0.002	0.0
	GT 39	25.8	0.45	0.77		GT 39	0.0	0.015	0.0
	GT 14	27.9	0.48	0.82		GT 33	0.3	0.057	0.05
1948	BT 8′	10.8	0.152	0.81	1953	I	0.7	0.037	0.20
II	GT 36	14	0.152	1.07	1953	GT 29	2.2	0.005	4.4
	GT 26	7.3	0.115	0.68	II	GT 4	2.2	0.03	0.73
1949	I	37*	1.00	0.59		GT 33	0.0	0.009	0.0
	II	20.8	0.49	0.53		BT 36	0.0	0.004	0.0
1950	GT 13	24	0.34	0.92	1954	I	0.2	0.005	0.47
I	GT 33	50	0.45	2.22	1954	GT 36	0.0	0.030	0.0
	GT 38	18	0.19	1.15	II	GT 22	1.5	0.045	0.32
1951	GT 33	2	0.27	0.07		GT 24	1.0	0.042	0.24
II	GT 34	2.5	0.154	0.17	In first two column:				
	GT 28	1	0.075	0.13	I = First broods				
	GT 36	2.7	0.067	0.42	II = Second broods				

of this point to be profitably attempted. However, it should be stressed that, as far as they go, the data for the other species (except for one understandable exception (*Diprion frutetorum* and *virens*, see p. 331)) all show the same *pattern* of deviation. This is additional support for the view that these deviations from expectation are a real phenomenon.

As for *Acantholyda*, we base this discussion on the data for separate broods (fig. 21 and table x), so as to give a fair idea of the scatter. For each brood we have a figure for the percentage of *Acantholyda* in the food, and one for its density in the respective territory[1].

For each separate observation we have calculated the value of $S = (R_O D_O/R_A$ (table x), and tested whether there is a correlation between this quantity and D_{Acanth}. Of course, S is here calculated from P_{Acanth} and D_{Acanth}, and hence is certainly not independent of D_{Acanth}. This influence, however, would result in a positive correlation between the two quantities compared. Our data, in contrast, show a negative

[1] For some periods it was impossible to estimate density in the separate territories, and then we had to us an average figure. In some of these cases, the census had been made over the whole area of the Zwarte Berg, and not in separate territories. In some other cases, twig sampling had yielded no prey in part of the territories, and thus only an estimate of the average density in all territories examined was possible.

The figures for 1948 I and II include data on 5 broods of Blue Tits (*cf.* p. 290).

correlation ($P \sim 0.0214$ by KENDALL's rank correlation method). This cannot possibly be ascribed to the dependency signalled above. Therefore, this way of checking our results must be considered reliable, if not sensitive, and the conclusion is permitted that the relative intensity of predation on *Acantholyda* is less at low than at higher densities of this prey.

In conclusion, we can state with some confidence that the deviations from the expectation curves reflect a real phenomenon.

3. THE INFLUENCE OF D_O

If, for a given prey species, it proves impossible to find an expectation curve that satisfactorily fits the whole set of observations, it follows at once that either D_O, or R_O/R_A, or both, must have varied among observations.

We shall first examine whether the deviations from expectation in our data can be attributed in their entirety to variations in D_O. In that case, there would be no reason to regard R_O and R_A as other than constant so that the data collected would support the probability of encounter hypothesis.

The equation for P_A:

$$P_A = \frac{100}{1 + \frac{R_O}{R_A} \cdot \frac{D_O}{D_A}}$$

proves the intuitively obvious point that, at given constant values of the risk indexes, the percentage of A in the food depends on the ratio of the density of A to that of all other food. If, for instance, D_O is relatively high, P_A will be small.

In fact, we have already discussed this influence of D_O on P_A on p. 304, and we have then indicated certain precautions by which we can restrict its manifestation. These precautions have also been observed in the arrangement of our data for fig. 21. Thus, the figures for *Panolis* have been divided into two series: one for periods in which acceptable alternative prey was present and one for periods in which this was lacking. In the same way, the data for *Cacoecia* before the appearance of large *Panolis* have been separated from those thereafter. Further, the data for *Cacoecia* in May 1951 have been excluded because the density of other species of prey, especially Syrphid larvae, was extremely high in this year (table II). In spite of these precautions, it cannot be expected that the influence of variations in the density of other species of prey is fully eliminated. Hence, it will be necessary to examine its effects in each separate case as far as possible.

The series for *Acantholyda* is the most complete one and, therefore, is considered first. In comparison with the best fitting expectation curve, this species formed low percentages in the food in 1951 II, 1952 I, 1952 II, 1953 I and 1954 II, but high percentages in 1948, 1949, 1950 and 1953 II. Was the density of other food higher in the former group of years than in the latter? It can be seen from table II that it was not. Adding together the densities of all kinds of food except *Acantholyda* as listed in this table for the periods involved, we find:

$P_{Acanth.}$	high:	Year	1948	1949	1950	1953 II	
		D_o	1.70	5.12	2.46	0.79	
$P_{Acanth.}$	low:	Year	1951 II	1952 I	1952 II	1953 I	1954 II
		D_o	1.63	0.88	0.89	1.29	?

Clearly, D_o did not tend to be higher in years with a low percentage of *Acantholyda* in the food. In fact, the available data suggest that the opposite might be true, but the difference is not significant.

It can be concluded that other factors than variation in D_o must be responsible, at least in part, for the deviation of the observed *Acantholyda* percentages from the expectation curve. In other words, R_o/R_A is not constant.

For the other species represented in fig. 21, less data are available, but it can at least be stated that they provide no argument that the situation here differs from that in *Acantholyda*. Thus, in *Cacoecia* (May), D_o for the one P_{Cac} that exceeds expectation has a value intermediate to that of the D_o's for the two P_{Cac}'s that remained below expectation.

Similarly, the D_o's for the three low *Diprion sertifer* percentages are not different from those for the two high ones (*viz.*, 0.66; 0.52; 0.41 and 0.98; 0.35, respectively). In the case of *Panolis* ("no alternative", fig. 21), the density of other food was much higher (1.27) when P_{Pan} was far above expectation than when it was equal to or below expectation (D_o = 0.65; 0.36; 0.41). Moreover, in June 1953 and June 1952 (when predation on *Panolis* was highest), there was an appreciable population of *Bupalus* and *Atolmis* moths (not mentioned in table II), whereas during the other observations these food species were not available. In the case of *Panolis* in the presence of an appreciable population of acceptable alternative prey, the density of total food except *Panolis* (2.15) when predation was below expectation was not higher than in the years when it was equal to expectation (6.10; 2.01 and 1.50, respectively).

On the whole, it seems safe to conclude that, although variation in D_O may have contributed to the scatter of the points plotted, the *pattern* of deviation from expectation (which appears to be the same in all but one type of prey) must be attributed to the fact that R_O/R_A is not independent of D_A, in contradiction to the hypothesis of probability of encounter.

4. THE INFLUENCE OF CHANGES IN RISK

Although changes in the value of R_O/R_A may be due to variation in either R_O, or R_A, or both, it is of course highly probable that the changes correlated with D_A discussed here will mainly be due to variation in R_A. This is not meant to imply that variation in D_A will never entail changes in R_O. In fact, it seems plausible in the light of

our data that changes in D_A may well make themselves felt in minor changes in R_O. These, however, will probably be of smaller extent and less regular in occurrence than the primary influence of D_A on R_A. Hence, the explanation of the observed deviations from expectation must in the first place be sought in variations in R_A.

In other words, the observations lead to the conclusion that both at low and at high D_A, R_A must be small in comparison with its value at intermediate densities of A. How can this be explained?

5. THE LOW RISK AT LOW DENSITIES

The remarkably low risk at low densities is perhaps most easily explained by the hypothesis of searching images. We have assumed that specific searching images are adopted only when the species in question has exceeded a certain density. Accordingly the birds would make only a limited number of chance encounters when density is below the critical level, while predation would become more efficient at higher densities. The observations on *Acantholyda*, *Cacoecia* and *Panolis* (without alternative) suggest that the critical density is rather sharply defined, for the increase in risk seems to be restricted to a rather narrow range of densities. The observations, however, are too few to warrant a definite conclusion on this point.[1]

It has already been mentioned that this increase in risk is found at different densities in different species. The critical density is low in *Panolis*, intermediate in *Acantholyda* and *Cacoecia*, and probably high in *Diprion sertifer*. This sequence is correlated with the specific indexes for risk. It is certainly a reasonable assumption that a highly appreciated prey provokes the acceptance of the searching image at much lower densities than an unpalatable one. Hence, this difference in critical density does not contradict the explanation presented here.

It should be remembered that the green *Diprion* larvae (*virens* and *frutetorum*) did not show a decrease in risk at low densities. We may explain this by assuming that the birds never used a searching image for these species during our observations, and that their capture was merely the result of chance contacts. In fact, the percentages recorded for the green *Diprion* larvae were lower than those for all other species

[1] In *Acantholyda* where risk seems to increase very suddenly when density becomes greater than 0.15, this impression depends on the fact that consumption was very low in 1951. It is possible that this was accidental. In the first place, predation pressure on *Panolis* was very low in this year. This points to abundant food. This might have prevented the tits from accepting *Acantholyda* as a searching image, though they would have done so had food in general been scarcer. Secondly, a number of dead, diseased *Acantholyda* were found on the twigs beside the living specimens (to which the density figure refers). This may have deterred the tits from collecting *Acantholyda*.

studied in this section. The circumstance that they always occur at the same time as *Panolis* may account for the apparent reluctance of the birds to adopt them as a searching image. This is not incompatible with the fact that the less palatable *Diprion sertifer* can be the object of a searching image, since that was seen only in periods of food scarcity, and *sertifer* disappeared from the diet as soon as *Panolis* had developed.

Finally, we may ask why specific searching images are not used at low densities. From a functional point of view, this can be understood if we assume that the birds can adopt only a limited number of searching images. Under these circumstances it will be more profitable to search for abundant than for rare prey. Moreover, it may be supposed that a certain number of encounters is required for the acceptance and maintenance of a searching image.

6. THE LOW RISK AT HIGH DENSITIES

Risk at high densities shows a decrease as compared to risk at moderate densities. This would imply that the searching image is used less when density is more than moderate. The observations even suggest that the birds do not allow the percentage to increase over a certain critical level, unless they cannot find an acceptable alternative prey. This level is low in the unpalatable *Diprion sertifer*, and higher in *Panolis* and *Acantholyda*. We may suppose that this "buffering" of the percentage in the food is the consequence of a preference for a mixed diet. Obviously, the birds concentrate on other species as soon as a certain kind of prey tends to predominate in their environment. This disposition probably has survival value. It is well known that many captive birds thrive better on a mixed diet than on a monotonous one. It is likely that this is true of tit nestlings as well.

XIII. DISCUSSION

The comparison of food composition and prey populations has led us to the hypothesis that the intensity of predation depends to a great extent on the use of specific searching images. This implies that the birds perform a highly selective sieving operation on the visual stimuli reaching their retina. This is an amazing ability, especially in the complex environment of needles and twigs where the tits do most of their hunting. As far as we are aware, the nervous mechanism of this process is not known.

The adoption of these searching images by the birds seems to be a process of conditioning, which takes place rather soon after the development of a new species of prey. There is some reason to believe that the birds can use only a limited number of different searching

images at the same time. Otherwise, it would be difficult to understand, why they do not form a searching image when the species in question is scarce.

Further, the tits can use a certain searching image with varying "intensity". When we consider the density-consumption curve for *Acantholyda* (fig. 21), we find that all points referring to densities under 0.10 can be fitted more or less into one particular expectation curve (the lower dotted line). In other words, at these low densities the birds either did not use a searching image at all (as we believe), or they used one always to the same extent. At densities of about 0.3, the percentage of *Acantholyda* in the food rose far above this expectation curve. However, it does not follow another expectation curve (*e.g.* the upper dotted line in fig. 21) from here on, but soon begins to fall behind. The latter effect is even more clear in *Diprion sertifer* and in *Panolis* (with alternative). This implies that at high densities the birds use their searching images to a smaller extent than at moderate densities.

Obviously, a number of different external factors determine the intensity with which a searching image is used. As far as we can see, the following properties of prey are important: size, conspicuousness, palatability and density of the species, and moreover, though to a smaller extent, the same properties of the other species present. Individual tits do not react to these factors in the same way. Sometimes they show remarkable examples of specialisation. Most probably this variability is caused by individual differences in preference as well as by chance. In the following discussion, we shall disregard these differences and we shall base our comments on the reactions of the population as a whole.

1. *Size.* This factor has been sufficiently discussed in the discussion of specific risk (p. 294). For example, the *Panolis* searching image was not acquired before the larvae had passed a certain stage of growth.

2. *Conspicuousness.* We believe that *Larentia firmata* was never accepted as a searching image because it is very hard to find. N. Prop (in this volume) was able to prove that its palatability is high. Probably its small size has an influence as well. Well-concealed large species of prey certainly give rise to a searching image.

3. *Palatability.* We found no evidence that the birds acquire a searching image for the unpalatable larvae of *Diprion pini*, although this species is extremely conspicuous. Further, *Diprion nemoralis* is eaten much less than *Acantholyda*, but is easier to find, at least for us. Here again, the searching image was used less in the less palatable species.

4. *Density.* The complex influence of this factor is discussed on p. 301

and p. 323. Evidently, there is an optimum density at which the searching image is used with the greatest intensity.

These factors can interact to a certain extent. The observations discussed on p. 331 show that palatable species of prey are already accepted as searching images at low densities, whereas in unpalatable species this occurs only at much higher densities. One might say that the former are attractive because they are tasty, and the latter because they are easy to get.

It is probable, therefore, that the acceptance of a searching image depends on the combined influence of all factors mentioned above.

What are the consequences of the principle of "specialised searching"? As regards the insect populations, its effects will be discussed in pt. II. A few words may be said, however, about its consequences for the birds, although these are difficult to evaluate. We cannot yet solve the problem whether the use of a specific searching image increases the over-all efficiency of food collecting, although it does increase the captures of the particular type of prey for which the searching image has been acquired. The answer to this question depends on whether or not the use of the searching image induces a "single-mindedness" in the bird, which causes a decrease in the yield of its hunting on the other types of prey.

One detail, however, seems clear. Under certain conditions non-selective searching would lead to a rather more monotonous diet than results from the use of specific searching images. This would be the case especially when one of the species of prey is much more abundant than the others. Most probably this effect has a certain survival value.

The use of specific searching images can therefore be regarded as one example of the high perfection in the food collecting methods of tits and other insectivorous birds, a perfection that might indeed be expected in view of the tremendous difficulties these birds have to overcome: Their prey generally have a very low density and are diluted in a tremendous amount of needles and twigs. Several of the more attractive species are very well camouflaged. And, finally, the daily ration which a parent tit must collect for its young is considerable.

XIV. SUMMARY

This paper deals with the factors which determine the composition of the food of tits (especially the Great Tit, *Parus major*) in pinewoods during spring and summer. Techniques are described for measuring density of insects and birds, and assessing food consumption by birds.

The relations between availability of insects and their occurrence

in the birds' food are discussed for a number of Lepidopterous and Hymenopterous species (chiefly larvae). The percentage in the food, formed by individual prey species, proved to be very different in different kinds of prey. The season of occurrence of each type of prey influences this percentage (together with the seasonal periodicity in general food density). Of the other factors determining the percentage in the food, special attention is paid to the "risk index" (definition, see p. 293). There is a strong correlation between risk and size, which depends, among other things, on the birds' dislike for a small prey. Some large and conspicuous species have a very low risk index. There are indications that these are unpalatable. The figures obtained do not show a clear correlation between risk and cryptic properties, but arguments are presented that the latter do tend to lower the risk index.

When a new species appears in the environment, its risk is low at first, and then increases suddenly. A detailed examination of this phenomenon leads to the hypothesis that tits when searching for prey concentrate on one or a few species at a time, and that, by a kind of learning process, they adopt "specific searching images" for these species. The main factors which determine whether or not this process will take place are discussed on p. 332. Among these, density of the prey species is important. It was found that the relation between the density of a prey species and its percentage in the food cannot be explained from probability of encounters alone. At low densities, consumption is lower than would be expected on that basis. At moderate densities it is unexpectedly high, and at high densities it falls again below expectation.

The difference in risk between low and moderate densities is explained by assuming that the birds do not adopt a specific searching image for a species of prey that is scarce. The decrease in risk at high densities is supposed to be due to the fact that, in order to obtain a sufficiently varied diet, the birds stop using a searching image when the species concerned forms more than a certain critical percentage in the total food.

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APPENDIX

by

L. DE RUITER

Professor TINBERGEN completed the manuscript of part 1 of this paper during the winter of 1954–1955. The results reported he regarded only as a first step toward the solution of the problems raised, and he intended to carry the analysis as much farther as would prove feasible in the field. Therefore, all observations were continued on the usual scale during the breeding season of 1955.

Now on one point there seemed to be a major discrepancy between the results obtained in that year, and those of all previous seasons. The comparison of the number of caterpillars in the twig samples with that of their faecal pellets revealed an unusually high rate of grass production (table 1). As fig. 1 shows, the inclusion of these data made the correlation between population density and faeces production much less good in both *Panolis* and *Acantholyda*. In fact, no significant correlation was demonstrable in *Acantholyda* any more.

Estimates of density are a cornerstone of the argument of TINBERGEN's paper, and many of these estimates are based on frass production alone.

TABLE I

Numbers of larvae in twig samples, and frass production during sampling period (compiled by Mr. L. M. Schoonhoven)

	Date	Larvae per sq.m.	Pellets per 0.25 sq.m. p. week
Panolis	VI 1948	0.13	7.0
	VI 1952	0.25	22.0
	VII 1952[1]	0.50	39.5
	VI 1953	0.06	5.3
	VII 1953[1]	0.43	30.0
	VI 1954	0.05	3.0
	VII 1955	0.27	37.3
Acantholyda	1948	0.49	?[2]
	VI 1949[1]	1.00	35.5
	1952	?[2]	?[2]
	VI 1953	0.04	0.48
	VI 1954	0.04	0.04
	VII 1955	0.20	36.5

[1] For reasons unknown to us, Tinbergen has plotted in fig. 4 of part I values slightly different from those reconstructed by us from the original data.
[2] We have not been able to reconstruct the data for this period.

Hence, the reliability of the correlation found in previous years was a matter of vital importance.

Tinbergen died soon after he had noted this discrepancy, and before he could add a discussion of the problems it raises to his manuscript. Such a discussion is clearly indispensable. Therefore, with the aid of Mr. P. J. Kuyten and Mr. L. M. Schoonhoven, who took part in the field work, I have attempted to fill this gap.

It should be pointed out first, that, even if we discard from the data all density estimates based on frass production alone, the remaining evidence still suggests that the main conclusion of Tinbergen's paper may be justified. The value of P for the conclusion that the risk index is higher at moderate densities than at low ones then becomes $P = 0.056$, which only slightly exceeds the conventional limit of significance ($P = 0.05$).

However, arguments will now be presented that the unusual results obtained in 1955 are due to abnormal conditions prevailing in that year, and not in other years of the investigation. If these arguments are valid, it is justified to exclude the 1955 data, and the discrepancy cannot affect the conclusions reached for the other years.

The faecal pellet counts leading to the detection of the discrepancy were made in August, after the return of Tinbergen and his team from

the field. It was not till then that anybody realised that the 1955 data differed in any way from those for previous years.

However, during the 1955 field season, TINBERGEN and his collaborators had often commented on the unusually cold and wet weather they experienced during April, May and June of that year, and on the influence of these inclement conditions on the insect populations studied. Never before in the course of the observations reported had TINBERGEN seen such a general retardation of the development of the insects.

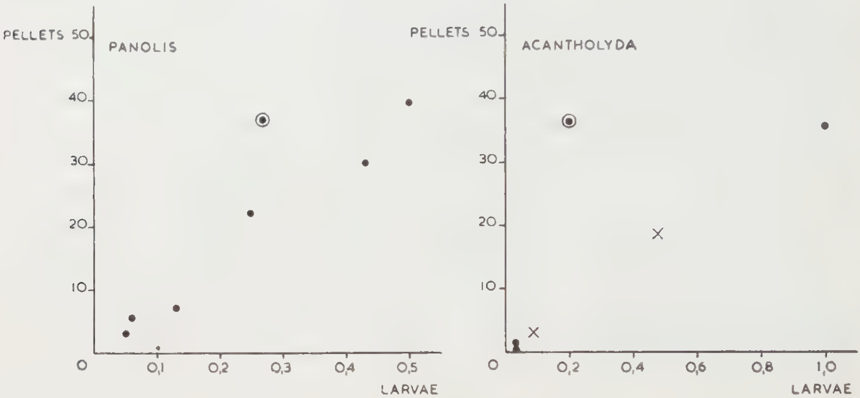


Fig. 1. Relation between frass counts and densities of larvae in twig samples for *Panolis* and *Acantholyda*, including the data for 1955 (marked with ⊙). Ordinates: average numbers of faecal pellets (diameter over 0.6 mm) per 0.25 sq.m. per 7 days. Abscissae: density of *Acantholyda* (> 10 mm long), or of *Panolis* (> 20 mm long), respectively, expressed as number of specimens per square meter of woodland. Values taken from TINBERGEN's original field data, (except those marked with ×, which are taken from fig. 4 of part 1 of TINBERGEN's paper)

At last, in the beginning of July, the weather improved markedly, so that it became about normal for the time of the year, although to the team in the field it seemed much milder because of the contrast with the foregoing period. At the same time, the rate of development of the insects increased very strongly, and, quite unexpectedly, most species eventually completed their cycles at about the normal dates.

Now the essential point is that the twig samples and frass samples compared were taken *in this period of accelerated development*. Obviously, this might be the explanation of the exceptionally high rate of faecal pellet production during the '55 sampling period.

In other words, there is some ground for the assumption that the discrepancy may be due to the fact that in 1955 insect development has been influenced by a factor which did not operate in the other years of the observations. In that case, it would be justified to disregard the data for 1955 in the discussion of those other years.

Of course the unanimous opinion of a team of competent observers, based on very close experience of the process concerned, carries considerable weight. Nevertheless it should be investigated whether the available quantitative data do confirm their impression that in 1955 insect development was unusually slow at first and exceptionally rapid later on, and, if so, whether this can be attributed to one or more environmental factors operating only in 1955.

Fortunately, although we cannot give a quantitative picture of the rate of insect development in general, quantitative evidence is available on the point most relevant here, viz. on frass production. These data suggest that the rate of faecal pellet production was unusually high during July 1955.

TABLE II

Acantholyda

1953		1954		1955	
Date	Total number of pellets per 0.25 sq.m.	Date	Total number of pellets per 0.25 sq.m.	Date	Total number of pellets per 0.25 sq.m.
8/V	1.3	27/V	0.2	7/VI	2.0
7/VI	5.3	11/VI	6.5	16/VI	5.9
17/VI	17.8	23/VI	13.6	24/VI	13.7
25/VI	23.8	1/VII	16.4	6/VII	25.7
1/VII	30.9	9/VII	23.5	20/VII	194.7
10/VII	38.3	20/VII	62.5	29/VII	351.1
17/VII	45.2	29/VII	95.6	4/VIII	388.0
28/VII	50.9	6/VIII	127.5	11/VIII	398.6
		16/VIII	147.5	24/VIII	404.4
		30/VIII	152.2	5/IX	408.6

Panolis

1954		1955	
Date	Total number of pellets per 0.25 sq.m.	Date	Total number of pellets per 0.25 sq.m.
11/VI	1.4	7/VI	0.4
23/VI	6.1	16/VI	0.4
1/VII	29.2	24/VI	3.2
9/VII	55.6	6/VII	20.1
20/VII	91.3	20/VII	154.9
29/VII	126.4	29/VII	264.9
6/VIII	195.1	4/VIII	308.6
16/VIII	246.4	11/VIII	340.1
30/VIII	261.9	24/VIII	359.0
		5/IX	364.4

We have checked this by comparing the time course of the faeces production of *Panolis* and *Acantholyda* in 1955 with that in 1954 (and, in the case of *Acantholyda*, 1953), these being the only years for which sufficiently extensive series of observations are available. The results are given in table II. For each species and each year, this table contains the total number of faecal pellets produced per $^1/_4$ sq.m. from the beginning of the season up to the successive dates mentioned in the first column for each year.

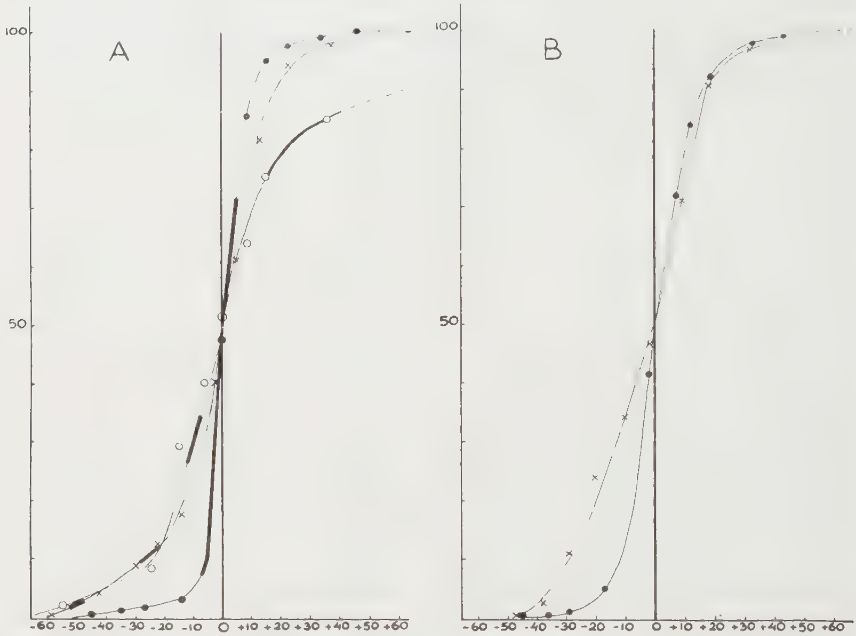


Fig. 2. Time course of frass production. A: *Acantholyda*, (heavy parts of curves indicate periods during which the twig samples were taken); B: *Panolis*. O—O: 1953; ×—×: 1954; ●—●: 1955. Further explanation in text

Fig. 2 represents the same data in a more easily comparable form. Here, the total number of faecal pellets (over 0.6 mm long) produced up to a given date is expressed as a percentage of the total pellet production per $^1/_4$ sq.m. during the whole life of the generation. Moreover, the day on which 50% of the total production had been achieved has arbitrarily been taken as the zero point on the time axis for all generations. The rate of faecal pellet production at a given time can be seen from the slope of the graph at the point concerned.

Two points stand out clearly from these graphs: 1. in both species, faecal pellet production was slower at first in 1955 than in 1954 (and 1953); 2. later on, the rate of production increased very much in 1955,

so that for some time it became steeper than at the comparable stages in other years. This confirms the impression gained by the field observers that the time course of insect development in 1955 was unusual.

We must now consider whether this was due to a combination of factors realised only in 1955. In view of the field workers' experiences, it is obvious to regard the climate as a possible cause. Little is known about the details of the influence of climatic fluctuations on insect development. It is certain, however, that very low temperatures retard growth. Further, caterpillars do not feed when their food plants are wet. In a wet season, therefore, growth will probably be slow. Finally, it seems probable that strong winds may hamper the movements of the larvae, and thereby cut down the amount of feeding they can do. This, again, may retard development.

With these points in mind, we have analysed the monthly surveys of weather conditions published by the K.N.M.I. (Royal Dutch Meteorological Institution) for the springs of 1948–1955 (fig. 3). The results can be summarised as follows:

a. Temperature. The effect on development of a given temperature has been found, within certain limits, to be in good approximation proportional to the product of its height and its duration. From the average temperatures for each ten-day period, we have calculated these products for individual intervals of that length, and plotted their sum as a function of time for each year separately in fig. 3 B. It is striking that during May and June the curve for 1955 is lower than any of the others. In July, however, the difference tends to disappear.

b. Moisture. The total amount of rain fallen was markedly higher in May and June 1955 than in any other year. Again, the difference is levelled in July. However, it would not be justified to conclude from this alone that during May and June '55 the food plants have been wet for longer periods than in other years. This depends on several other factors as well, e.g. on the humidity of the atmosphere, on the duration of the rainfall, and on the temperature of the foodplants. Now the total number of hours of sunshine was not different in 1955 from that in other years (fig. 3 D). This suggests: a. that the duration of the rainfall may not have differed very greatly among years; b. that the temperature of the vegetation (which depends in part on absorbed radiation) will not have been abnormally high in 1955. Neither was the relative humidity of the atmosphere in May and June 1955 different from that in other years. Hence, it is very likely that the rate of evaporation will not have varied greatly, so that the food of the larvae will have been wetter in 1955 than in other seasons, due to the greater quantity of rain.

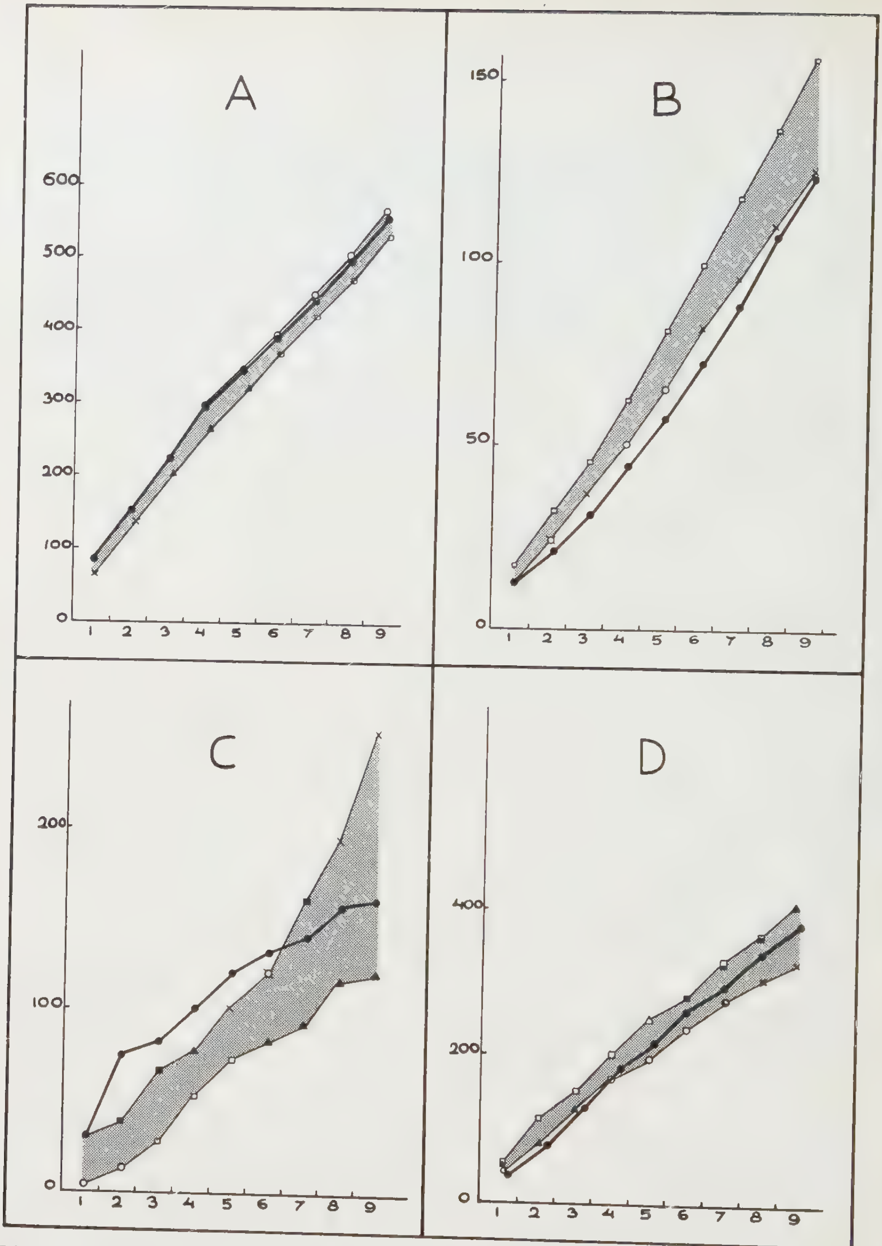


Fig. 3. Climatic conditions in the various years of the investigation. A. relative humidity (cumulative graph of products of humidity × duration); B. temperature (cum. gr. of temp. × duration); C. rainfall (in mm, cum. gr.); D. sunshine (hours, cum. gr.). Abscissa: time (1 = first ten days of May, 9 = last ten days of July). In each graph conditions in 1955 (●—●) are compared with the limits between which the factor concerned varied in 1949–1954. In the latter period conditions were so similar always that curves representing individual years had to be omitted for the sake of clarity. Clearly, 1955 was exceptionally cold and wet. Further explanation in text.

c. "Windiness" (expressed as the sum of the products for individual 10-day periods of wind velocity and duration) was higher in 1955 than in other years. Here, however, the agreement among the other years is less good.

In view of the above the statements seem justified, 1. that in May and June of 1955 climatic conditions have been such that the development of the insects may have been considerably retarded as compared with all other years studied, and 2. that no important differences existed in this respect among the other years.

The fact that in July 1955 insect development was much accelerated, although weather was not better than normal suggests several interesting problems. It is as though the populations wanted to make up for time lost. However, at the present state of our knowledge, a discussion of this point could hardly be fruitful.

One thing, however, seems beyond doubt. The discrepancy in the rate of faeces production between 1955 and other years was due to an abnormally rapid development of the insects in 1955, caused by a combination of climatic factors realised only in 1955, all other years of the investigation being rather similar in climate.

Therefore, the correlation between insect density and frass production in the earlier years should be based only on data collected in those years. The data for 1955 must be treated separately.

THE NATURAL CONTROL OF INSECTS IN PINE WOODS

II. CONDITIONS FOR DAMPING OF NICHOLSON OSCILLATIONS IN PARASITE-HOST SYSTEMS

by

L. TINBERGEN AND H. KLOMP

I. INTRODUCTION

It has been shown in part I of this study that the intensity of predation by birds on insects in pine woods depends on the population density of the prey. However, the relationship between the two is not such as to guarantee stability of the prey populations. At low density of a prey, it is true, the predators will not acquire a searching image for that species, and thus their grip on its population will be loosened. This may help the prey to return to more normal levels. However, at abnormally high prey densities, the predators become increasingly reluctant to take that prey. Neither can the predator population increase in numbers so rapidly as to put a check on the escaping prey population. Hence, in a system consisting only of such prey and such a predator, control of the prey density would be far less adequate than is the case in nature. Evidently, other factors take a share in regulating the prey population in nature. We shall consider some of these factors, and their interaction in this part of the paper.

Parasites are an obvious choice for careful consideration in this connection. Moreover, important theoretical controversies have arisen around the problem of the role of parasites in the natural regulation of populations.

In 1933 NICHOLSON has published a paper in which he presents a number of arithmetic calculations on the influence of entomophagous parasitoids on the population density of their hosts. These calculations were presented as examples to show the principles of balance and the role of competition in the natural regulation of animal numbers.

This paper has been criticized by several authors (e.g. THOMPSON 1939, 1956; MILNE 1957). As a rule the objections are raised against NICHOLSON's (1933) ideas about competition and the role of competition in natural control. Moreover, authors do not agree with several premisses of the arithmetic models, such as a constant power of increase of the host and an invariable searching activity of the parasite. VARLEY (1947) has emphasized the ever-increasing amplitude of the oscillations to be the main objection which may be levelled against the theory. He argues that under natural conditions many parasite-host systems are not characterized by this phenomenon.

Reading this amount of objections and criticism one might easily get the idea that NICHOLSON's theory on parasite-host interaction is largely wrong. The main purpose of this paper is to show that after modification of NICHOLSON's premisses and after their adaptation to more natural conditions many of his original ideas and starting points can be maintained and used in a working hypothesis.

Briefly the premisses of NICHOLSON can be summarized as follows. He starts with the assumption that the host produces a fixed number of progeny (power of increase = P.o.I.), while the fecundity of the parasite is unlimited. The number of parasite eggs deposited out of this huge supply depends on host density, because the parasites search at random and the probability of finding a host is proportional to host density. The capacity of the parasite to find its host is referred to as the area of discovery (= A.o.D.) and may be defined as the area effectively searched during the lifetime. The size of this area is constant and independent of host density due to the fact that the searching activity of the parasite is uninfluenced by host density. Therefore, the area of discovery of the parasite is an index for the keenness of its senses and its efficiency in capture.

If one parasite individual is searching in a definite habitat the area of discovery is an index for the proportion of parasitized hosts. When several parasites search in that habitat the sum of their areas of discovery (called the *area traversed* (A.T.) by the parasite population) is not proportional to the percentage of parasitism. For, with increasing parasite density, the areas traversed (= the areas of discovery) by different individuals overlap more and more, and there is a growing competition to find hosts not yet parasitized by other parasites. Therefore, the graph showing the relationship between fraction parasitized (= *area covered*, A.C.) and parasite density (expressed as the area traversed by the total parasite population = parasite density times area of discovery) is named the *competition curve* (fig. 1).

Because the parasites search at random the relation between area

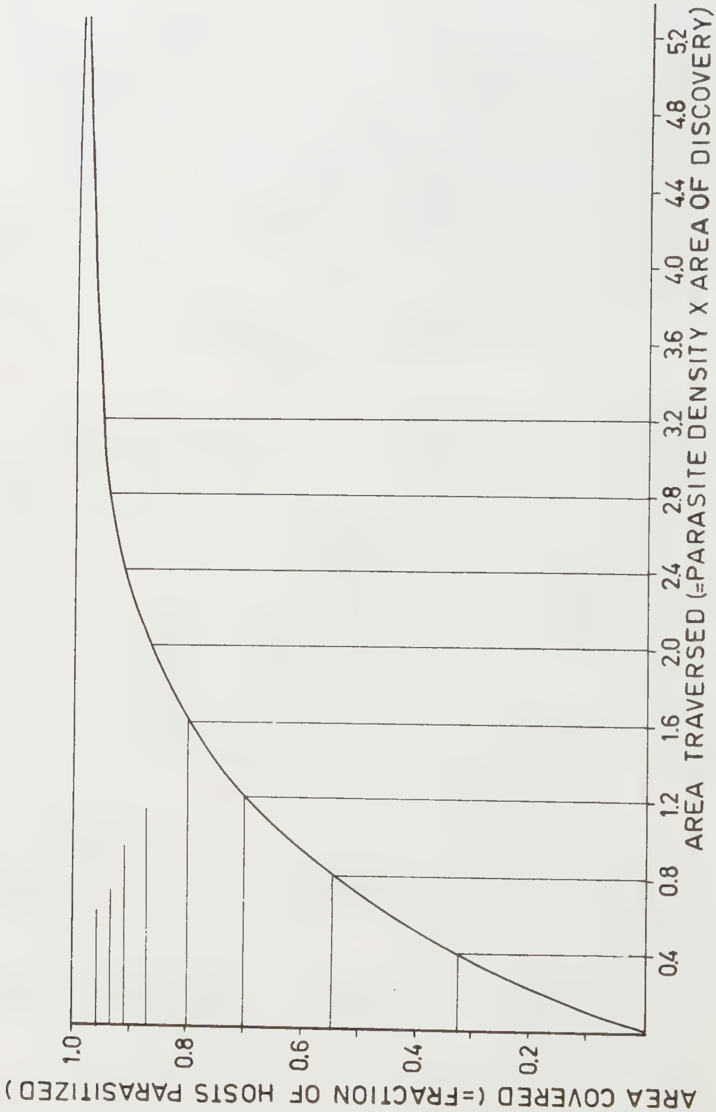


Fig. 1. The competition curve of NICHOLSON.

traversed and area covered can be expressed mathematically as follows (see: NICHOLSON and BAILEY 1935)

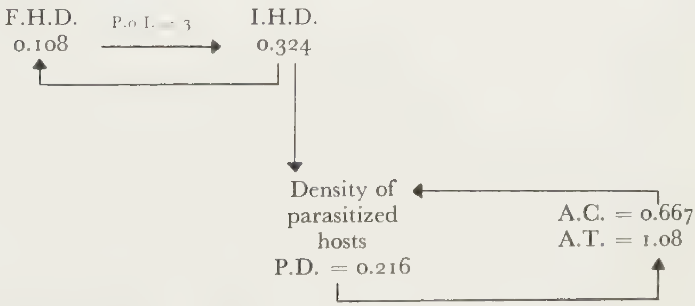
$$\text{area covered} = 1 - e^{-\text{area traversed}} \tag{1}$$

With this formula the fraction of hosts parasitized can be calculated when the parasite density and the area of discovery are known.

Assuming definite values for the power of increase and the area of discovery NICHOLSON computes a so called steady state (S.S.). Then, the population is in a state of stationary balance, because the surplus offspring of the host is destroyed by the parasite. When one host individual can only provide food for the development of one parasite, the calculation of the steady densities (S.D.) runs as follows (*cf.* NICHOLSON 1933, p. 145):

A.o.D. = 5; P.o.I. = 3; 1 host produces 3 offspring, and of these one must survive, so that the percentage of parasitism is 66.7%, and the area covered = 0.667. From formula (1) follows: area traversed = 1.08 (*cf.* fig. 1). This area traversed is the sum of all individual areas of discovery and, therefore, the parasite density (= P.D.) = 1.08/5 = 0.216. Because the parasite has no mortality the P.D. is the same as the number of parasitized hosts. Hence, the initial host density (= I.H.D.) = 3/2 × 0.216 = 0.324.

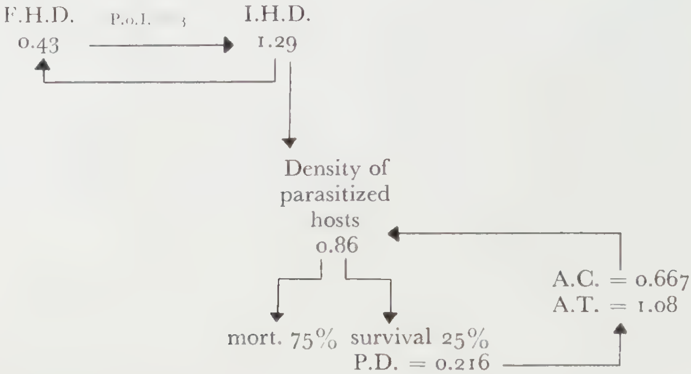
In diagram:



It is very unlikely that under field conditions the parasites would have no mortality. Therefore we shall introduce such a mortality factor in the model. As has already been shown by NICHOLSON the steady density of the parasite is unaltered by this introduction, but that of the host is increased. When 75% of the adult parasites is killed, the calculation of the steady state is as follows. Because the same density of mature parasites is required in order to find and destroy the surplus

of hosts, the P.D. = 0.216, that is 25% of the number of parasitized hosts. Hence, the initial host density, I.H.D. $= 3/2 \times 4/1 \times 0.216 = 1.29$.

In diagram (*cf.* S.S. in table 1):



Under natural conditions, where the population is influenced by a number of varying factors the steady state will not be maintained. Nicholson has shown, however, that any departure from the steady density sets up a reaction that tends to cause a return to this density. We shall give a numerical example of this reaction.

We assume in the foregoing model that host density is twice its steady value due to, say, a weather influence. Because the parasite is still at its steady density it takes 66.7% of the population and gives rise to $0.25 \times 0.667 \times 2.58 = 0.43$ adult parasites in the second generation (P.D. in table 1). Hence, the parasite increases in numbers and in the second generation a greater fraction of hosts (89%) is eliminated. The I.H.D. in the second generation can easily be calculated. The number of non-parasitized hosts is $0.333 \times 2.58 = 0.86$ (F.H.D.) and, hence, the I.H.D. = F.H.D. $\times$ P.o.I. $= 0.86 \times 3 = 2.58$. In the same way the densities of host and parasite of all later generations can be computed (see table 1). The values have been plotted in fig. 2.

As will be seen from fig. 2 the densities of the interacting animals oscillate about their steady values with increasing amplitudes. This last fact can be explained in this way. The density of the parasite is a result of the host density in the preceding generation. Due to this delay in effect the parasite is still very numerous even though the host has decreased in numbers and, thus, host density falls far below the steady density and this in turn, causes a great reduction in parasite numbers. The host then recovers again, but, due to the scarcity of the parasite, will reach much higher numbers than in the previous oscillation.

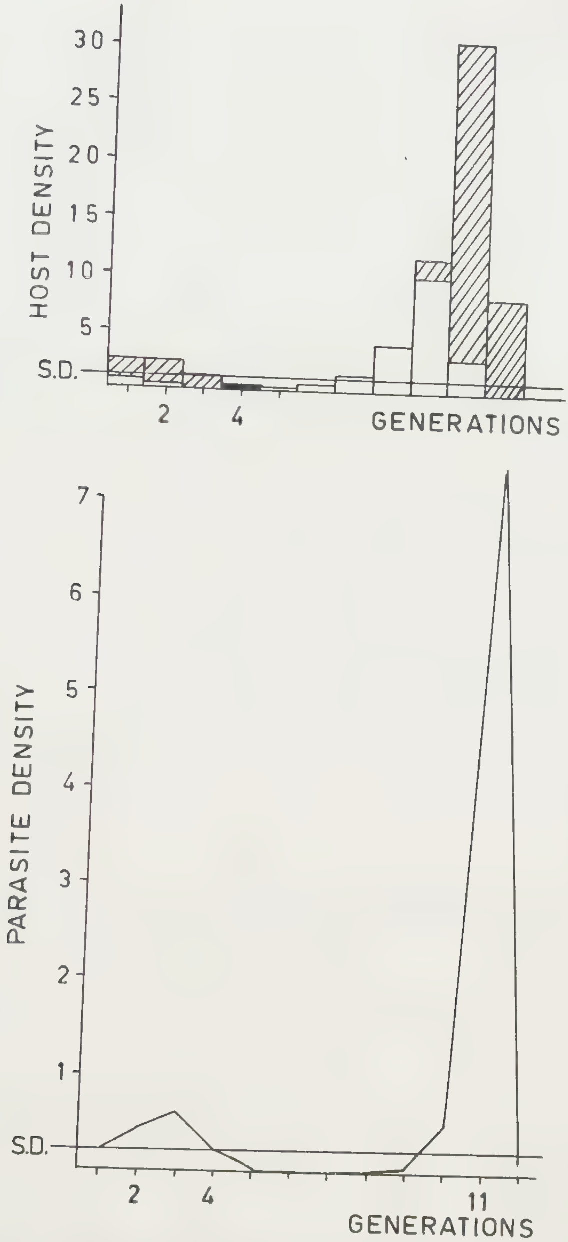


Fig. 2. Oscillations in a simple host-parasite model. P.o.I. = 3, A.o.D. = 5. Parasite mortality 75%. Starting deviation of I.H.D. = 2 × S.D. *Cf.* table 1. The hatched blocks indicate the fraction of hosts parasitized in each generation.

Nicholson claims that oscillation with increasing amplitudes sooner or later must lead to densities much below their steady values (*cf.* table 1 and fig. 2). The animals then will be irregularly distributed in small groups, the positions of which are continually changing. His conclusion is based on the following argument. Increasing oscillation ultimately reduces host and parasite to very low densities "...In the next generation it is clear that the animals must be scattered in the environment in more or less widely separated groups, for each female forms a centre from which its offspring diffuse. Some of these groups are found by parasites, and it is likely that these groups will be exterminated during the next few generations... The other groups of hosts increase geometrically for a few generations, but are sooner or later found by parasites and are ultimately exterminated; in the meantime there has been a migration of hosts, some of which have established new groups. Thus, instead of there being a simultaneous oscillation of the animals throughout the whole environment, there are large numbers of independent local systems of oscillation, all phases of oscillation being represented in the environment at any given time. The fact that parasites can develop only in areas in which hosts occur means that such areas are searched more intensively than the rest of the environment. Consequently, the parasites do not have to cover a fraction of the whole environment equal to the fraction of hosts that is surplus, but only need to cover a much smaller fraction in order to find the surplus hosts. Therefore the density of parasites, and consequently the density of hosts, necessary for balance under the given conditions is much below the calculated values of the steady densities of the animals".

TABLE I

Oscillations in a simple host-parasite model. P.o.I. = 3, A.o.D. = 5. Parasite mortality 75%. Starting deviation of I.H.D. = 2 × S.D. (*Cf.* fig. 2)

Gen.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	P.D.	A.T.	A.C.
S.S.	1.29	66.7	0.43	0.86	0.216	1.08	0.667
1	2.58	66.7	0.86	1.72	0.43	2.15	0.89
2	2.58	89	0.28	2.30	0.575	2.87	0.94
3	0.84	94	0.05	0.79	0.20	1.00	0.63
4	0.15	63	0.06	0.09	0.02	0.10	0.10
5	0.18	10	0.16	0.02	0.005	0.025	0.025
6	0.48	2.5	0.47	0.01	0.003	0.013	0.013
7	1.41	1.3	1.39	0.02	0.005	0.03	0.03
8	4.17	3	4.04	0.13	0.03	0.16	0.16
9	12.12	16	10.18	1.94	0.49	2.43	0.91
10	30.54	91	2.74	27.8	7.45	35.3	0.999

However, fieldwork has revealed that quite a number of hosts neither show such patchy distribution nor are characterized by oscillations with increasing amplitudes. NICHOLSON, being possibly aware of this difficulty, has given a model with damped oscillations (p. 164). In this example the parasite attacks another host species in addition to the one it controls. When the density of the controlled host is higher than the steady density the violence of the increase of the parasite is reduced, for the density of the parasite is also in part determined by its other host, the density of which remains unchanged. When this reduction of parasite increase is relatively great the effect leads to damping of the oscillations, which are set up by the increase of density of the controlled host.

However, this only holds when the influence of the parasite on the additional host is counteracted by the regulating mechanism of this host. When the parasite is numerous owing to large numbers of the first host, it is unlikely, that the regulating mechanism of the second host will be able to counterbalance the high mortality due to the parasite. Therefore, this mechanism of damping does probably not function in natural populations.

To sum up, the host-parasite models of NICHOLSON based on animals with a constant area of discovery and power of increase are characterized by oscillations with increasing amplitude when the steady state is once disturbed. In theory this ultimately leads to a patchy distribution pattern which is not realized under natural conditions.

A host-parasite system with a regular distribution of the host is only possible when the oscillations are damped. A mechanism with this effect proposed by NICHOLSON cannot be accepted owing to the fact that it must break down in natural populations.

It is evident that the starting points of NICHOLSON give rise to host-parasite systems, which contradict the facts found under natural conditions. Therefore, it is logical to bring NICHOLSON's initial assumptions more in accordance with the experience of field workers. First, it has long been recognized that the factors kept constant by NICHOLSON will vary irregularly under the influence of the ever-changing field conditions. Moreover, the properties of the animals as P.o.I. and A.o.D., but also parasite mortality as introduced in the above example, may be density dependent. Again, the systems will be more complex and include e.g. other mortality factors of the host, which may be either density dependent or independent.

In the following chapters the influence of these factors will be studied. Chapter II deals with a fluctuating weather factor in a simple parasite-host model. In chapters III and IV the influence of a density dependent

P.o.I. and A.o.D. is discussed. Chapter v is devoted to a complex system in which a Nicholson host parasite model is combined with a density dependent mortality factor and this leads to damped oscillations. Finally, chapter vi deals with the introduction of favourable meteorological conditions, which suddenly raise host density. It is shown that this can give rise to infestations, in particular in a model, the oscillations of which are damped by bird predation.

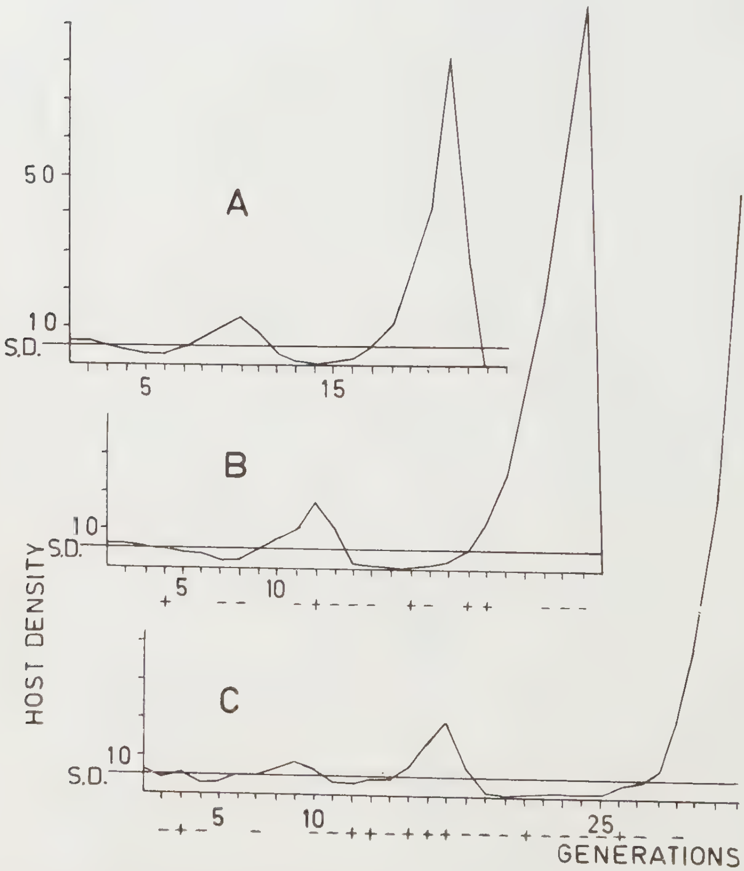


Fig. 3. Oscillations in a host-parasite model under the influence of a fluctuating weather factor. P.o.I. = 2 in normal years, = 2.5 in favourable (+) and = 1.5 in unfavourable years (—). A.o.D. = 1. Parasite mortality 75%. Starting deviation of I.H.D. = 1.25 × S.D. *Graph A:* Oscillations with P.o.I. constant. *Graphs B and C:* Oscillations with fluctuating P.o.I. *Cf.* table ii.

II. THE INFLUENCE OF A FLUCTUATING WEATHER FACTOR

In the examples of host–parasite interaction given by NICHOLSON all factors are kept constant and this has been a source of considerable criticism. It is argued by several authors that this cannot be in nature because of the ever-changing influence of the weather. Therefore, it is reasonable to see which is the effect of such a variable weather factor.

In a simple host-parasite model as discussed on p. 348 the effect of a fluctuating weather factor can be realized in three ways: by an influence on parasite mortality, parasite activity or on the power of increase of the host. We have chosen the last method and the model is constructed as follows. In normal years with average weather conditions the P.o.I. = 2, changing into 2.5 during favourable and into 1.5 during unfavourable years, assuming that host and parasite have one generation a year. Favourable, normal and unfavourable years occur at random. To get the weather type prevailing during a definite generation a random choice was made out of a box containing three times ten marbles of three different colours representing the types of weather.

TABLE II

Oscillations in a host-parasite model under the influence of fluctuating weather conditions. P.o.I. in normal years 2, in favourable years 2.5 (+) and in unfavourable years 1.5 (—) A.o.D. = 1. Parasite mortality 75%. Starting deviation of I.H.D. = 1.25 × S.D. *Cf.* fig. 3C

Gen.	P.o.I.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	P.D.	A.T.	A.C.
S.S.	2	5.44	50	2.72	2.72	0.68	0.68	0.50
1		6.80	50	3.40	3.40	0.85	0.85	0.58
2	—	5.10	58	2.14	2.96	0.74	0.74	0.52
3	+	5.35	52	2.57	2.78	0.70	0.70	0.50
4	—	3.85	50	1.93	1.93	0.48	0.48	0.39
5		3.85	39	2.35	1.50	0.38	0.38	0.32
6		4.70	32	3.19	1.51	0.38	0.38	0.32
7	—	4.69	32	3.18	1.51	0.38	0.38	0.32
8		6.36	32	4.33	2.03	0.51	0.51	0.46
9		8.66	46	4.68	3.98	1.00	1.00	0.63
10	—	7.02	63	2.60	4.42	1.11	1.11	0.67

Further, the calculations are based on an A.o.D. = 1 and a constant parasite mortality = 75%. The steady state of this model is given in table II. The oscillations are introduced by bringing host density on 1.25 times the steady density. With constant P.o.I. = 2 the oscillations run as shown in fig. 3A.

We have made two sets of random weather conditions. Favourable and unfavourable years are indicated in the figs. 3B and 3C with + and — signs. All other generations have met normal weather conditions. In table II we have only given the first ten generations of set 3C.

As can be seen from a comparison of graph 3A with B and C even this pronounced influence of the weather has no principal effect on the host-parasite interaction. In fact the peaks are somewhat higher and occur some generations later, but this is due to the random succession of favourable and unfavourable years.

III. THE INFLUENCE OF THE DENSITY DEPENDENCY OF THE POWER OF INCREASE

It is quite evident that a density dependency of the fecundity (P.o.I.) of the host as shown in fig. 4, where the fecundity decreases linearly with increasing host density, must have a damping influence on the fluctuations in a host-parasite model. For, compared with a constant

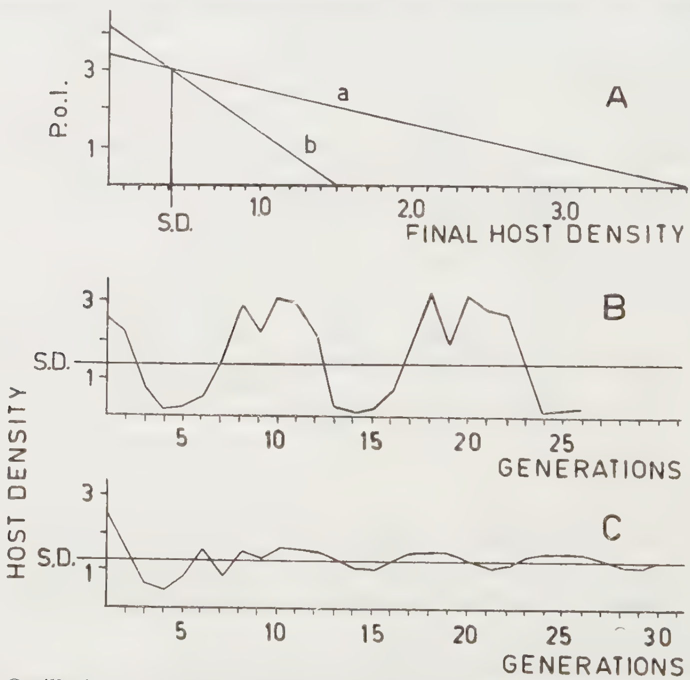


Fig. 4. Oscillations in a host-parasite model with P.o.I. density dependent. The steady state and fluctuations with constant P.o.I. have been given in table I and fig. 2. When the density dependency of the P.o.I. is as shown by graph a, the amplitudes of the oscillations are nearly constant (B). When the P.o.I. decreases as shown by graph b, the amplitudes are weakly damped. *Cf.* table III.

P.o.I., the host will not increase so quickly to high densities when the parasite is scarce, and hence, the recovery of parasite numbers will be less impetuous.

The damping effect will be slight when the density dependency of the P.o.I. is weak. In this case the amplitude of the fluctuations may increase slowly or remain constant. Fluctuations with decreasing amplitude will only occur with very strong density dependency of the P.o.I. Examples of these effects have been given in fig. 4. The density dependencies of the P.o.I. applied in these models are shown in

TABLE III

Oscillations in a host-parasite model with P.o.I. density dependent corresponding to the relationship given in fig. 4A, graph b. Steady state as in table 1. Starting deviation of I.H.D. = 2 × S.D. *Cf.* fig. 4C

Gen.	P.o.I.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	P.D.	A.T.	A.C.
S.S.	3	1.29	66.7	0.43	0.86	0.216	1.08	0.667
1	—	2.58	66.7	0.86	1.72	0.43	2.15	0.89
2	1.8[1]	1.55	89	0.17	1.38	0.35	1.72	0.82
3	3.7	0.63	82	0.11	0.52	0.13	0.65	0.48
4	3.9	0.43	48	0.22	0.21	0.05	0.25	0.22
5	3.6	0.79	22	0.62	0.17	0.04	0.20	0.18
6	2.4	1.49	18	1.22	0.27	0.07	0.35	0.31
7	0.7	0.85	31	0.59	0.26	0.07	0.35	0.31
8	2.5	1.48	31	1.02	0.46	0.12	0.60	0.45
9	1.3	1.33	45	0.73	0.60	0.15	0.75	0.52
10	2.1	1.53	52	0.73	0.80	0.20	1.00	0.62
11	2.1	1.53	62	0.58	0.95	0.24	1.20	0.70
12	2.6	1.51	70	0.45	1.06	0.27	1.35	0.75
13	2.9	1.31	75	0.33	0.98	0.25	1.25	0.72
14	3.3	1.09	72	0.31	0.78	0.19	0.95	0.61
15	3.3	1.04	61	0.41	0.63	0.16	0.80	0.54
16	3.0	1.27	54	0.58	0.69	0.17	0.85	0.58
17	2.5	1.45	58	0.61	0.84	0.21	1.05	0.65
18	2.5	1.52	65	0.53	0.99	0.25	1.25	0.72
19	2.7	1.43	72	0.40	1.03	0.26	1.30	0.73
20	3.0	1.22	73	0.33	0.89	0.22	1.10	0.66
21	3.3	1.07	66	0.36	0.71	0.18	0.90	0.59
22	3.2	1.15	59	0.47	0.68	0.17	0.85	0.58
23	2.8	1.33	58	0.56	0.77	0.19	0.95	0.61
24	2.6	1.46	61	0.57	0.89	0.22	1.10	0.66
25	2.6	1.47	66	0.50	0.97	0.24	1.20	0.70
26	2.8	1.40	70	0.42	0.98	0.25	1.25	0.72
27	3.0	1.26	72	0.35	0.91	0.23	1.15	0.69
28	3.2	1.12	69	0.35	0.77	0.19	0.95	0.61
29	3.2	1.12	61	0.44	0.68	0.17	0.85	0.58

[1] When the F.H.D. = 0.86, the P.o.I. = 1.8, according to fig. 4A, graph b.

fig. 4A. The calculations of fig. 4C have been given in table III.

Thus, in theory, a strongly density dependent reproduction of the host results in damped oscillations. To be so, fecundity must fall greatly at density levels which are slightly above the mean density. In natural populations this effect has never been observed. Therefore, it is very unlikely that this mechanism of damping operates in actual fact. Cf. KLOMP 1958b.

IV. THE INFLUENCE OF THE DENSITY DEPENDENCY OF THE AREA OF DISCOVERY

One of NICHOLSON's basic assumptions is that parasites search at random and that the searching activity and, hence, the area of discovery (see p. 345) are constant and independent of host and parasite densities. This implicates that in non-selective parasites (see below) the number of eggs laid per female parasite is independent of parasite density and increases linearly with host density. This can be shown in the following way.

Non-selective parasites do not discriminate between parasitized and non-parasitized hosts. Consequently, the distribution of the eggs deposited is purely at random. The fractions of hosts with 0, 1, 2... etc. eggs can be calculated, therefore, with the Poisson formula, starting from different mean numbers of eggs per host (M):

$$e^{-M} \times \frac{M^x}{x!}, \text{ where } x = 0, 1, 2, \ldots \text{ etc.}$$

The fraction non-parasitized hosts is

$$e^{-M} \times \frac{M^0}{0\,!} = e^{-M}$$

and, hence, the fraction of hosts parasitized is: $1 - e^{-M}$. In the introduction we have seen that

$$\text{area covered} = 1 - e^{-\text{area traversed}}$$

and since the fraction parasitized hosts equals the area covered, follows M = area traversed, or in other words: the mean number of parasite eggs deposited per host equals the value of the area traversed in non-selective parasites.

Now, the number of eggs laid per parasite (N) can be derived as follows:

$$N = \frac{M \times D_H}{D_p} = \frac{\text{area trav.} \times D_H}{\text{area trav./}_{\text{A.o.D.}}} = \text{A.o.D.} \times D_H \qquad (2)$$

where D_H and D_p are host and parasite density, resp.

Because the area of discovery is constant, N is a linear function of D_H (*cf.* fig. 5, curve 1).

In selective parasites a host already parasitized before will be avoided. Therefore, all parasitized hosts bear one egg and the fraction of hosts infected (= area covered) equals the mean number of eggs per host. The number of eggs laid per parasite (N) can be computed as follows:

$$N = \frac{M \times D_H}{D_p} = \frac{\text{area covered} \times D_H}{D_p}$$

Thus, in selective parasites the number of eggs deposited per parasite is depending on host and parasite density. When the egg supply of the parasite is unlimited this phenomenon has no influence on the controlling capacity of the parasite as compared with non-selective parasites with the same area of discovery. Therefore, Nicholson had not to discriminate between these two types of parasites in his calculations. When the egg supply is limited, however, the selective parasites will be more effective, for at increasing parasite density the chance to meet a host already parasitized increases. These hosts are avoided in selective and infected again (without any result, see p. 347) in non-selective parasites. Therefore, the former distribute their eggs more economically and can reach higher percentages of parasitism than the latter.

In all parasite-host models dealt with here non-selective parasites are introduced.

It is unlikely, however, that the egg-supply of the parasite should be unlimited. (For arguments see VARLEY 1947, and KLOMP 1958a.) It is more logical to assume that at a given critical value of host density (C.D.) the egg amount of the parasite will be exhausted. This means

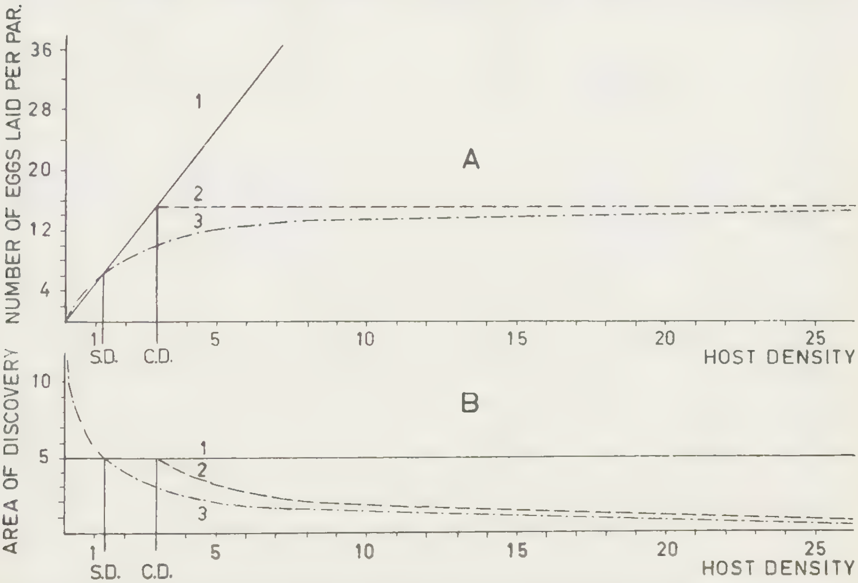


Fig. 5. A. Graph showing the relation between host density and the number of eggs laid per parasite (N). 1. The egg supply of the parasite is unlimited and the searching activity independent of host density. 2. The egg supply of the parasite is limited and amounts to 15 eggs. The whole amount is deposited at the critical (C.D.) and still higher host densities. 3. The activity of the parasite is slowed down with increasing host density and the number of eggs deposited approaches the maximum production asymptotically. B. Graph showing the relation between host density and the size of the area of discovery, applied to the three cases given in graph A. 1. The area is constant and independent of host density. 2. The area is density dependent at host densities higher than the critical level (C.D.). 3. The area is density dependent along the whole range of host densities. In 2 and 3 the size of the area of discovery approaches asymptotically to zero. The size of the area of discovery can be derived from graph A by means of formula (2); see the text.

that the area of discovery decreases with host density at values higher than the critical one (fig. 5).

The influence of this density effect on the parasite–host relationship can easily be predicted. For, in fact it means, that the searching activity of the parasite decreases at the higher densities of the host, *i.e.* at the very moment that a fast increase of the percentage of parasitism is wanted to prevent the host from reaching extremely high densities. Consequently, the phenomenon of the increasing amplitude will be strengthened. This effect is demonstrated in fig. 6, graph 2 and the calculations are partly (up to the 9th generation) given in table IV.

The activity of the parasite, however, not only may be limited by the total egg supply in this simple way. It might be, that the activity is slowed down temporarily after each successful egg deposition. This means that in addition to the density effect just mentioned the parasite

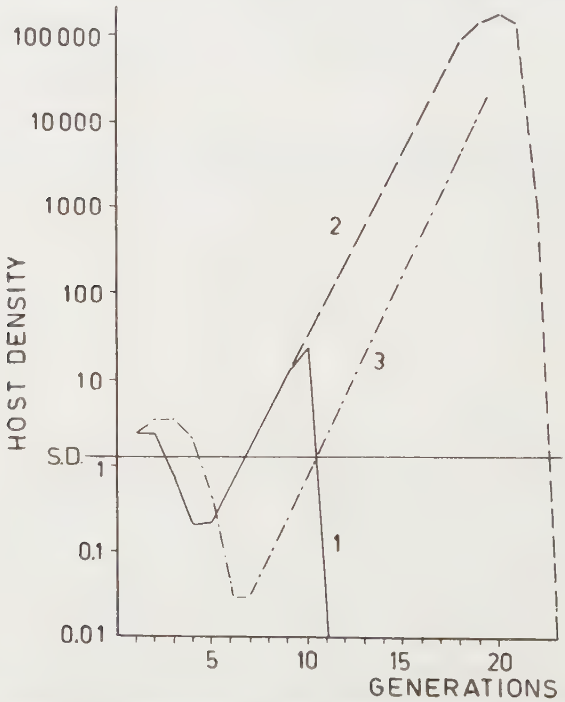


Fig. 6. Oscillations in a host-parasite model with the A.o.D. density dependent. P.o.I. = 3, A.o.D. = 5. Parasite mortality 75%. Starting deviation of the I.H.D. = 2 × S.D. *Graph 1:* egg supply of parasite unlimited and A.o.D. constant (*cf.* fig. 5 and table I). *Graph 2:* egg supply of the parasite limited and amounting to 15; A.o.D. density dependent at densities higher than the critical level (*cf.* fig. 5 and table IV). *Graph 3:* egg supply of the parasite limited and the activity of the parasite slowed down after a successful egg deposition (*cf.* fig. 5 and table V).

TABLE IV

Oscillations in a host-parasite model with the A.o.D. density dependent corresponding to the relationship in fig. 5, graph 2. Steady state as in table I. Starting deviation of I.H.D. = 2 × S.D. *Cf.* fig. 6, graph 2

Gen.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	P.D.	A.o.D.	A.T.	A.C.
	—	—	—	—	0.216	5	1.08	0.667
1	2.58	66.7	0.86	1.72	0.43	5	2.15	0.89
2	2.58	89	0.28	2.30	0.575	5	2.87	0.94
3	0.84	94	0.05	0.79	0.20	5	1.00	0.63
4	0.15	63	0.06	0.09	0.02	5	0.10	0.10
5	0.18	10	0.16	0.02	0.005	5	0.025	0.025
6	0.48	2.5	0.47	0.01	0.003	5	0.013	0.013
7	1.41	1.3	1.39	0.02	0.005	3.5[1]	0.017[2]	0.017
8	4.17	1.7	4.10	0.07	0.02	1.2	0.024	0.024
9	12.30	2.4	12.00	0.30	0.08	0.4	0.03	0.03

[1] A.o.D. = N/I.H.D. (*cf.* p. 356) = 15/4.17 = 3.5 (max. number of eggs = 15).
[2] Area trav. = P.D. × A.o.D. = 0.005 × 3.5 = 0.017.

TABLE V

Oscillations in a host-parasite model with the A.o.D. density dependent corresponding to the relationship given in fig. 5, graph 3. Steady state as in table I. Starting deviation of I.H.D. = 2 × S.D. *Cf.* fig. 6, graph 3

Gen.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	P.D.	A.o.D.	A.T.	A.C.
					0.216	3.6	0.78	0.54
1	2.58	54	1.19	1.39	0.35	3.0	1.05	0.65
2	3.57	65	1.25	2.32	0.58	2.9	1.68	0.81
3	3.75	81	0.71	3.04	0.76	3.9	2.96	0.94
4	2.13	94	0.13	2.00	0.50	7.7	3.85	0.98
5	0.39	98	0.01	0.38	0.095	10	0.95	0.61
6	0.03	61	0.01	0.02	0.005	10	0.05	0.05
7	0.03	5	0.029	0.0015	0.0004	8.2	0.003	0.003
8	0.09	0.3	0.085	0.0001	0.00002	7.9	0.0002	0.0002
9	0.25	0.02	0.254	0.00001	0.000002	6.1	0.00001	0.00001

is more active at very low than at intermediate densities of the host. Arguments which support this assumption can be found in the experimental results of DE BACH and SMITH (1941) and BURNETT (1951, 1954). In this case the area of discovery is density dependent along the whole range of host densities (fig. 5).

The influence of this density effect on the fluctuations in a host-parasite model is demonstrated in fig. 6, graph 3 and the calculations are partly (up to the 9th generation) given in table V. It is evident that the fluctuations are still more violent than in the foregoing case (fig. 6,

graph 2). For at low host densities the parasite is activated, the percentage of parasitism being higher than under Nicholson's conditions, and, consequently, the host will be reduced to still lower density levels. At densities higher than the steady density the activity of the parasite decreases and the host can escape to extremely high densities. Hence, density dependency of the area of discovery—which seems to be more in agreement with natural conditions—strengthens the increase of the amplitudes of the oscillations.

In our examples of fig. 6 we have represented an extreme case. In the steady state an individual parasite deposits 6.45 eggs on the average (see fig. 5) and its maximum production is 15 eggs. Under natural conditions the fecundity of parasites is generally much higher and, consequently, the density dependency of the area of discovery less extreme as in the example chosen.

V. THE INFLUENCE OF A DENSITY DEPENDENT MORTALITY FACTOR

A. MORTALITY OF THE PARASITE

In the parasite–host model under consideration we introduced a constant mortality of the parasite amounting to 75% (see p. 347). Under natural conditions such a mortality factor cannot be constant. It will either fluctuate irregularly when it is determined by density independent factors, or the fluctuations will be correlated with parasite density when the size of the mortality is depending on parasite numbers.

The influence of an irregularly fluctuating factor has already been studied by means of a weather factor influencing the P.O.I. of the host (chapter II). A density independent mortality of the parasite has the same principal effect.

The effect of a density dependent mortality of the parasite on the fluctuations inherent in the host–parasite model of Nicholson is considered in fig. 7. In graph A parasite mortality is assumed to increase linearly with increasing density.

In this graph parasite density as plotted on the abscissa is the density of parasitized hosts given in the tables. It may be assumed that all infected hosts give rise to parasite adults which then are exposed to the influence of the mortality factor.

When this type of mortality is introduced in the parasite host model of fig. 2 (table I) the phenomenon of the increasing amplitude is considerably weakened (fig. 7, B; table VI, up to the 13th generation). This effect can be strengthened in different ways, resulting in oscillations with decreasing amplitudes, *e.g.* by increasing the slope of graph A (fig. 7), or by combining density dependent parasite mortality with another factor with the same effect. This cooperation of two density dependent factors is demonstrated in fig. 7, C. The calculations are

TABLE VI

Oscillations in a host-parasite model with density dependent parasite mortality corresponding to the relationship given in fig. 7A. Steady state as in table I. Starting deviation of I.H.D. = 2 × S.D. *Cf.* fig. 7B

Gen.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	Parasite survival	P.D.	A.T.	A.C.
1	2.58	66.7	0.86	1.72	0.175[1]	0.30	1.50	0.785
2	2.58	78.5	0.56	2.02	0.147	0.30	1.50	0.785
3	1.68	78.5	0.37	1.31	0.210	0.28	1.40	0.75
4	1.11	75	0.28	0.83	0.253	0.21	1.05	0.64
5	0.84	64	0.30	0.54	0.273	0.15	0.75	0.52
6	0.90	52	0.43	0.47	0.277	0.13	0.65	0.47
7	1.29	47	0.68	0.61	0.270	0.16	0.80	0.55
8	2.04	55	0.92	1.12	0.227	0.25	1.25	0.71
9	2.76	71	0.80	1.96	0.154	0.30	1.50	0.785
10	2.40	78.5	0.52	1.88	0.161	0.30	1.50	0.785
11	1.56	78.5	0.34	1.22	0.218	0.27	1.35	0.73
12	1.02	73	0.28	0.74	0.259	0.19	0.95	0.61
13	0.84	61	0.33	0.51	0.280	0.14	0.70	0.50

[1] When the density of parasitized hosts = 1.72, the survival of the parasite is 17.5%, according to fig. 7A.

partly (up to the 10th generation) given in table VII. Here the combined effect of two factors, which operating separately do not damp the fluctuations effectively, leads to decreasing amplitudes.

B. MORTALITY OF THE HOST

It has already been discussed on p. 351 that natural host–parasite systems will include other mortality factors of the host in addition to the parasite. In this section we shall study the effect of the density dependency of such an additional mortality factor on the NICHOLSON oscillations.

Assuming the mortality of the host to increase linearly with increasing density it is easy to recognize after what has been said in the foregoing subchapter, that this can lead to damped oscillations (cf. p. 370). However, this type of linearly increasing mortality factor is purely theoretical. Part I of this study deals amply with the role of insectivorous birds operating as predators on caterpillars. Therefore, it is obvious to introduce this natural mortality factor in the host–parasite system.

First, the relation between percentage predation and the population density of the prey (host) will be briefly considered. In part I the effect of birds has always been expressed as the percentage occurrence in the food of a definite prey species. It has been shown that the observed relation between density and percentage contribution to the food

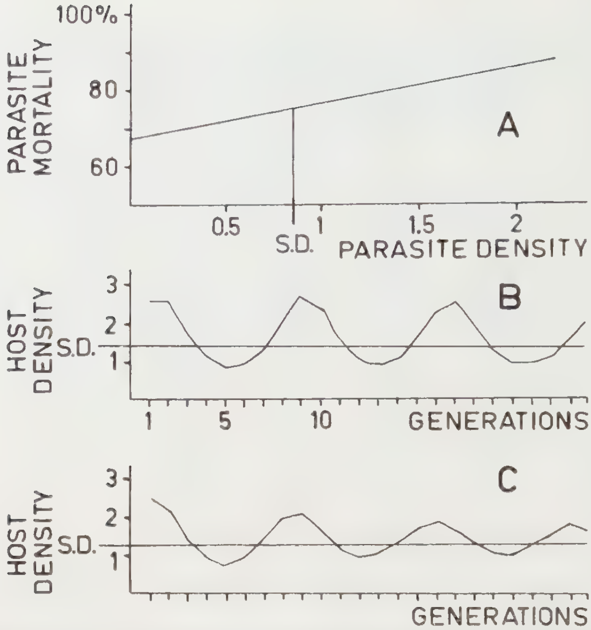


Fig. 7. Oscillations in a host-parasite model with parasite mortality density dependent. P.o.I. = 3, A.o.D. = 5. Starting deviation of the I.H.D. = 2 × S.D. For steady state and oscillations with constant parasite mortality see table I and fig. 2. *Graph B:* oscillations with parasite mortality density dependent according to graph A (*cf.* table VI). *Graph C* shows the damping effect of the coöperation of a density dependent parasite mortality (according to graph A) and a density dependent P.o.I. of the host (according to fig. 4A, graph a). For calculations see table VII.

TABLE VII

Oscillations in a host-parasite model with parasite mortality and P.o.I. of host density dependent corresponding to the relationships given in fig. 7A and 4A, graph a, resp. Steady state as in table I. Starting deviation of I.H.D. = 2 × S.D. *Cf.* fig. 7C

Gen.	P.o.I.	I.H.D.	% par.	F.H.D.	Density of parasitized hosts	Parasite survival	P.D.	A.T.	A.C.
1	—	2.58	66.7	0.86	1.72	0.175[2]	0.30	1.50	0.785
2	2.62[1]	2.25	78.5	0.48	1.77	0.170	0.30	1.50	0.785
3	2.97	1.43	78.5	0.31	1.12	0.227	0.25	1.32	0.74
4	3.13	0.97	74	0.25	0.72	0.261	0.19	0.95	0.61
5	3.17	0.79	61	0.31	0.48	0.283	0.14	0.70	0.50
6	3.13	0.97	50	0.485	0.485	0.281	0.14	0.70	0.50
7	2.97	1.44	50	0.72	0.72	0.261	0.19	0.95	0.61
8	2.74	1.97	61	0.77	1.20	0.219	0.26	1.30	0.73
9	2.70	2.08	73	0.56	1.52	0.193	0.29	1.45	0.76
10	2.90	1.62	76	0.39	1.23	0.216	0.26	1.32	0.74

[1] When F.H.D. = 0.86, the P.o.I. = 2.62, according to fig.4A, graph a.
[2] See note [1] of table VI.

deviates from the corresponding relation based on the probability of encounter hypothesis. (In this hypothesis it is assumed that the searching movements of the birds are random, that the distribution of the prey is random, and that the bird's preference for a definite prey species is independent of prey density.) It is evident that this deviation will also manifest itself in the relation between density and percentage predation of the population. The curve showing this relation based on the probability of encounter hypothesis can be derived by the following reasoning.

In the territory of a polyphagous bird several species of prey are available. We assume that 15% of the total prey population is composed of species A. In a certain year this species increases twofold in numbers, while the others together remain constant. Then, A is 100/115 × 30 = 26% of the population. If the risk index of species A is independent of A's density the bird now takes 26/15 of the quantity taken in the foregoing generation. The numbers of the prey increased to 30/15 of the quantity in the last year. Hence, the percentage predation decreases to 26/30 of the original value. Calculations of this type at several densities of species A—assuming bird density and the density of the other prey species together to be constant—reveal the decline in predation with increasing prey density given in fig. 8, curve 1.

It has been shown in part 1, however, that the risk index of a definite prey species varies with its density. The cause of this is twofold. First, the birds develop a searching image for prey species at intermediate densities and concentrate on them while foraging. Hence, they only take

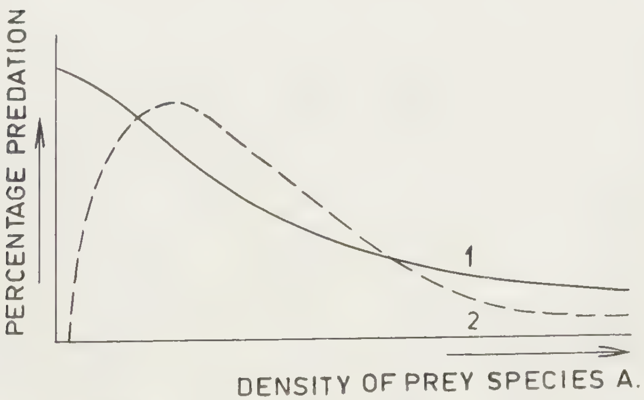


Fig. 8. Relation between percentage predation by a population of polyphagous birds and the density of a definite prey (caterpillar) species A. Bird population density and the density of all other prey species together occurring in the area, are assumed to be constant. Curve 1 gives the relation when the brid's preference for A is independent of the density of A. Curve 2 shows the emperically determined relation (see the text).

a relatively small fraction of the prey species which are at the lower densities. Secondly, the birds try to vary their diet and avoid species which are relatively very numerous.

As to prey species A this means that at intermediate densities the risk index is higher, and at low and high densities it is lower than the assumed constant value. In general, this results in a density dependent predation in the range from low to intermediate densities and in an inversely density dependency from intermediate to high densities of the prey (fig. 8). We shall see that this has far reaching consequences.

1. *The birds prey on parasitized and non parasitized larvae*

Now we construct a model with three main components: host (prey), predator (bird) and parasite, and we start with a predator taking parasitized and non-parasitized larvae of the host indiscriminately, being this probably the most common case (*cf.* part 1).

It is assumed that the predator takes 37.5% of the hosts in the steady state. It is evident that under field conditions this percentage varies with different prey species, depending a.o. on their conspicuousness and palatability. The value chosen in this model has been found in the field. It is rather high, however, and as a rule the fractions taken are lower (Tinbergen 1949, table 1). In the model presented here the P.o.I. of the host = 2, the A.o.D. = 1 and the parasite mortality = 75%. Then the steady state can be calculated as follows.

Starting from 10 host individuals (F.H.D.$_2$) the I.H.D. after reproduction is

TABLE VIII

Oscillations in a host-predator-parasite model. P.o.I. = 2, A. o. D. = 1. Predation by birds 37.5%. Parasite mortality 75%. Both mortality factors are constant. Starting deviation of I.H.D. = 1.25 × S.D. *Cf.* fig. 9A

	I.H.D.	% par.	F.H.D.$_1$	F.H.D.$_2$	Density of parasitized hosts	Dens. par. hosts after bird pred. P.D.$_1$	Density of surviving parasites P.D.$_2$	A.C.
S.S.	7.05	20	5.65	3.53	1.41	0.88	0.22[1]	0.20
1	8.81	20	7.05	4.41	1.76	1.10	0.28	0.25
2	8.81	25	6.61	4.13	2.21	1.38	0.35	0.30
3	8.26	30	5.78	3.61	2.48	1.55	0.39	0.32
4	7.22	32	4.91	3.07	2.31	1.44	0.36	0.30
5	6.14	30	4.30	2.69	1.84	1.15	0.29	0.26
6	5.38	26	3.98	2.49	1.40	0.88	0.22	0.20
7	4.98	20	3.98	2.49	1.00	0.63	0.16	0.14
8	4.98	14	4.28	2.68	0.70	0.44	0.11	0.095
9	5.36	9.5	4.85	3.03	0.51	0.32	0.08	0.075

[1] The column Area trav. is omitted because the A.o.D. = 1.

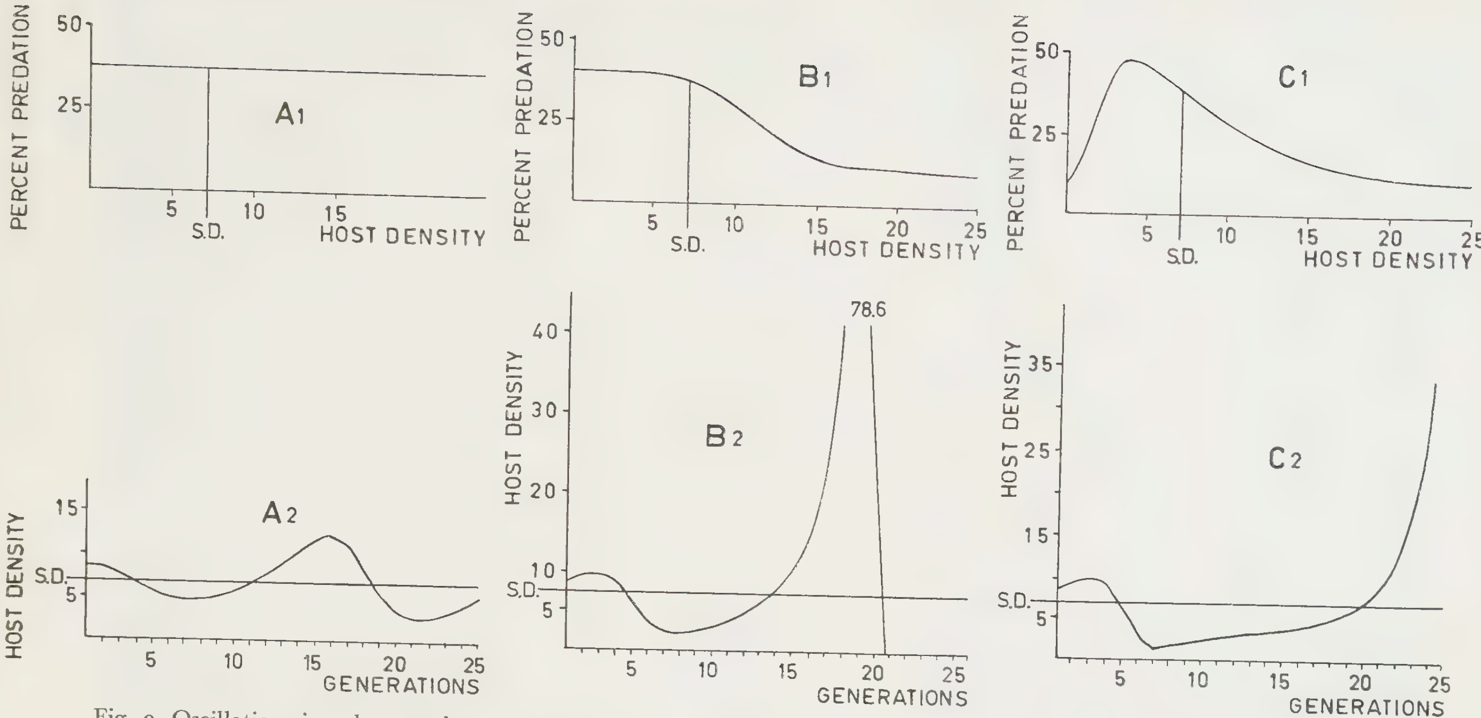
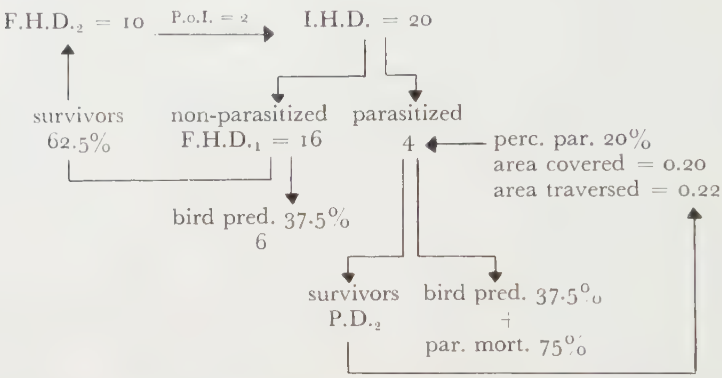


Fig. 9. Oscillations in a host-predator-parasite model. P.o.I. = 2, A.o.D. = 1. Parasite mortality 75%. Bird predation 37·5% in the steady state. Starting deviation of I.H.D. = 1.25 × S.D. *Graph A:* per cent predation constant (A1) and oscillations with increasing amplitudes (A2). *Cf.* table VIII. *Graph B:* per cent predation density dependent as shown in B1; the increase of the amplitudes of the oscillations considerably strengthened (B2). *Graph C:* per cent predation maximal in the range of lower host densities (C1); oscillations of the same type as B2.

10 × 2 = 20. These are partly parasitized and from the non-parasitized animals 62.5% survive the predator. This number of survivors equals the initial number in the steady state. Hence, the number of non-parasitized hosts (F.H.D.$_{,1}$) is 100/62.5 × 10 = 16 and, consequently, the number of parasitized hosts and the percentage of parasitism are 4 and 20%, resp. The parasites, which survive the predator and the mortality factor operating after the bird, have to cover 20% of the area and, then, as can be derived from the competition curve (fig. 1), the area traversed is 0.22. Because the A.o.D. = 1, the steady density of the parasite (P.D.$_{,2}$) amounts to 0.22/1 = 0.22 (*cf.* p. 345).

In diagram:



Starting from this parasite density the steady densities of the host (F. and I.H.D.) can easily be calculated with the above diagram. When the density of the surviving parasities is 0.22, the density of parasitized hosts amounts to 0.22/(0.625 × 0.25) = 1.41, and the I.H.D. = 100/20 × 1.41 = 7.05 (table VIII).

Giving the initial host density a deviation of 1.25 times the steady density, the fluctuations resulting from this disturbance have increasing amplitudes (fig. 9A; table VIII; host density as plotted in fig. 9A1 and corresponding figures is the I.H.D. That is the sum of parasitized and non-parasitized hosts, upon which the birds prey).

In this model we will now examine the effect of different types of density dependent mortality due to birds.

a. *The influence of a predator with non-selective searching.* The density dependency used in this model is given in fig. 9B1. After what has been said in the foregoing chapters it will be evident that this type of predation strengthens the increase of the amplitudes of the fluctuations (fig. 9B2).

b. *The influence of a predator with selective searching:* the optimal host density (the density with the highest percentage predation) being smaller than the steady host density. The density dependency used in this model is given in fig. 9C1. Although the mortality rate increases

strongly along a small range of the lower host densities, this range is too small to have a damping effect on the oscillations. After being reduced to a low density level by the parasites, the host slowly recovers to optimal density levels and, then, owing to the fall in bird predation, can quickly grow out to extremely high densities, the parasite being scarce due to the fact that the host density was lower than the steady density during a long range of generations (fig. 9C2).

c. *Like the foregoing model* (b), but optimal and steady densities coincide. The density dependency used in this model is given in fig. 10A1. Here the mortality rate increases along a greater range of host densities. It is to be expected that this will lead to a weak damping effect. For, in the foregoing example there is a small range of host densities (between optimal and steady density) where both bird predation and percentage parasitism decrease. In the model considered here the effect of predator and parasite on the host is always opposite. At increasing densities lower than the steady density bird predation grows and the number of parasites decreases; at densities higher than the steady host density it is just the reverse (fig. 10A1; cf. fig. 9C met 10A).

d. *Like model b* (p. 366), but the optimal host density higher than the steady density. The density dependency used in this model is given in fig. 10B1. Here the mortality rate increases along a still greater range and it will now be evident that this results in decreasing amplitudes of the oscillations (fig. 10B2). The calculations are partly (up to the 12th generation) given in table IX.

e. *Like model d*, but the disturbance of the steady density of the host 2.5 times instead of 1.25 times. As can be seen from fig. 10B3, the oscillations caused by this rather great disturbance are damped, as well.

In all the complex models mentioned so far the birds take 37.5% of the host in the steady state. This is a relatively considerable fraction of the prey population. In the field such a great influence of birds has only been found in some very attractive and relatively conspicuous caterpillars (TINBERGEN 1949). Cryptic prey species generaly run a lower risk. Therefore, we will now study the effect of 12.5% and 25% bird predation.

f. *Bird predation 12.5% in the steady state.* This necessarily makes the influence of the parasite greater, which amounts to 43%. This can easily be derived in a diagram as shown on p. 366. Where the parasite gets the mastery of the predator so much, it can be expected that the damping effect of the birds is relatively too small to counteract the disturbing influence of the parasite. This is shown in fig. 11A.

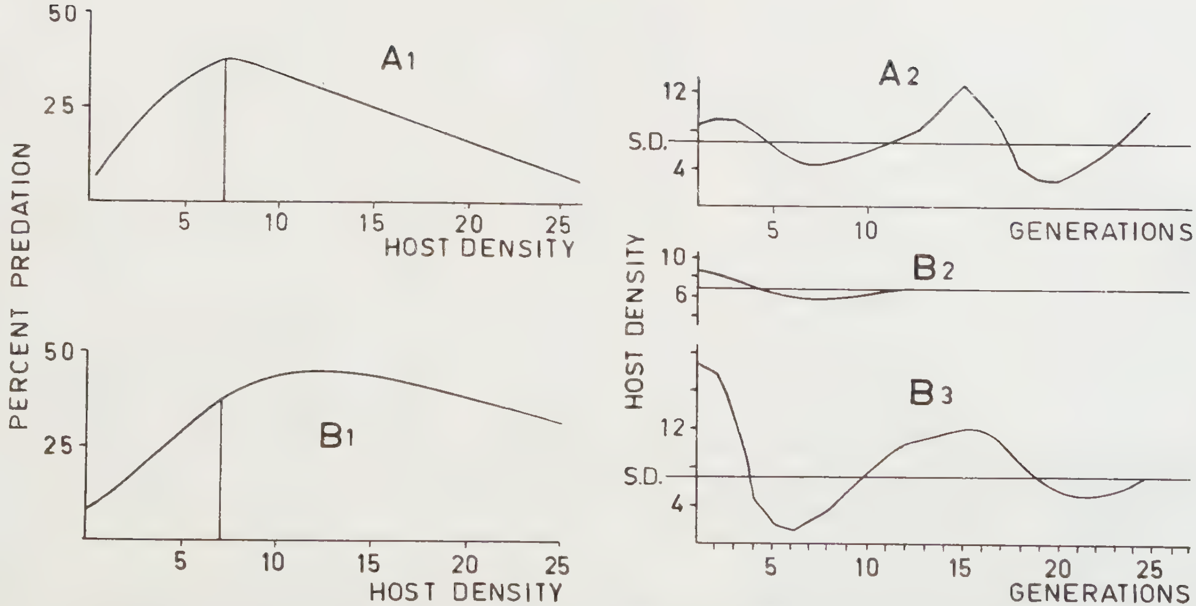


Fig. 10. Oscillations in a host-predator-parasite model. P.o.I. = 2, A.o.D. = 1. Parasite mortality 75%. Bird predation 37·5% in the steady state. Starting deviation of I.H.D. in graphs A2 and B2 = 1.25 × S.D., in graph B3 = 2.5 × S.D. *Graph A:* per cent predation maximal in the range of intermediate host densities (A1); oscillations with increasing amplitude, but rate of increase smaller than in the example of fig. 9C. *Graph B:* per cent predation maximal in the range of higher host densities (B1) and, consequently increasing along a large range; the oscillations are damped (B2). *Cf.* table IX. *Graph B3:* density dependency of percentage predation as in graph B1; disturbance of S.D. greater; the oscillations are strongly damped.

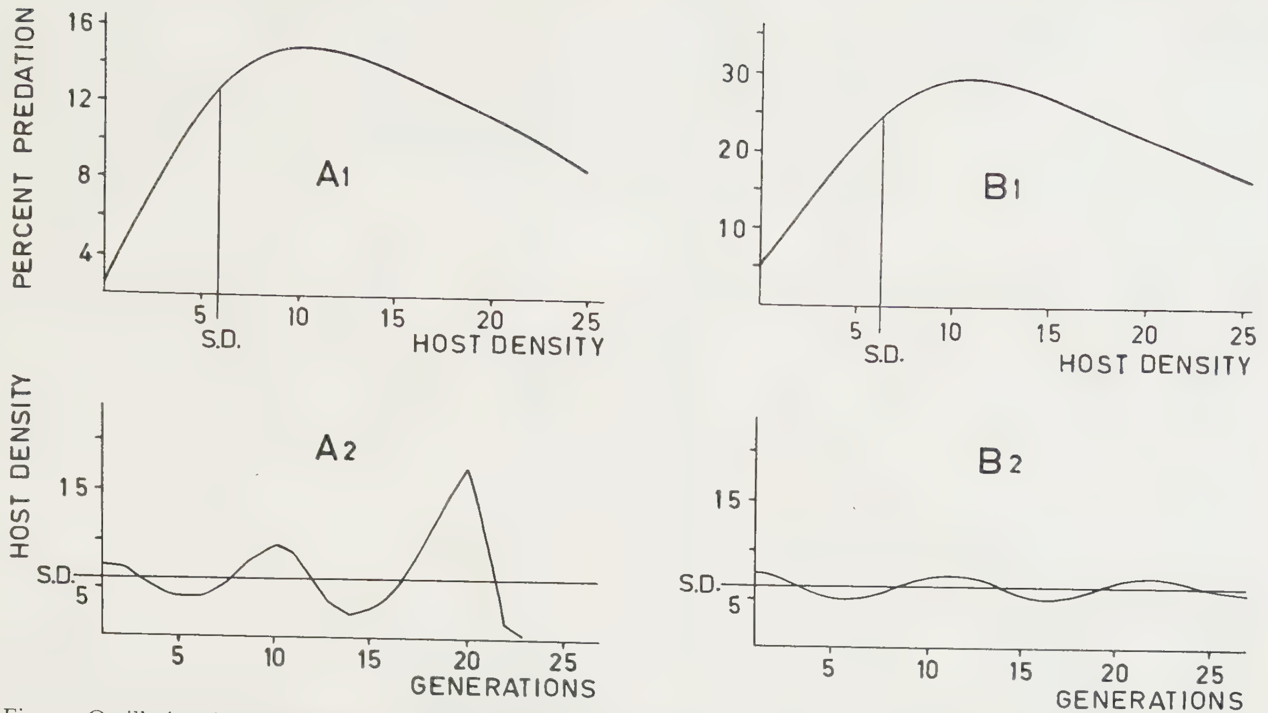
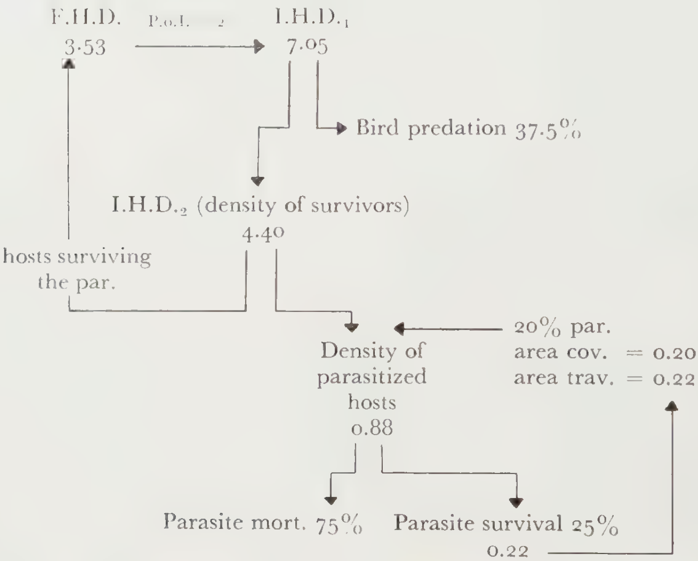


Fig. 11. Oscillations in a host-predator-parasite model. P.o.I. = 2, A.o.D. = 1. Parasite mortality 75%. Predation by birds in the steady state 12.5% and 25% in the models A and B, resp. Starting deviation of I.H.D. = 1.25 × S.D. *Graph A:* the influence of the predator is relatively too small; the parasite causes oscillations with increasing amplitudes. *Graph B:* the predator is able to counteract the disturbing influence of the parasite; the oscillations have weakly decreasing amplitudes.

2. *The birds prey on non-parasitized larvae*

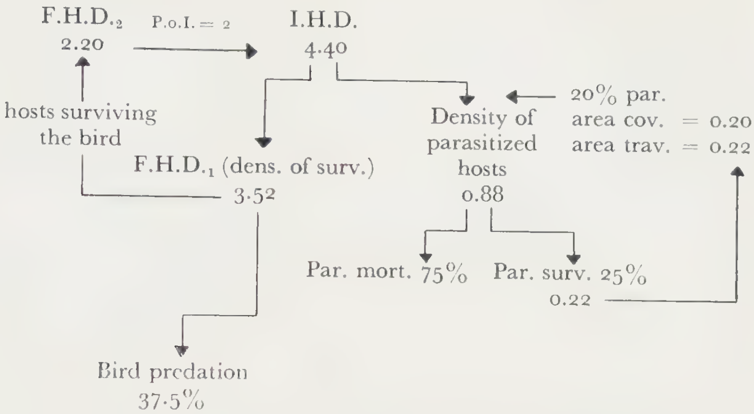
In the foregoing subchapter the birds preyed on parasitized and non-parasitized hosts indiscriminately. When certain conditions are fulfilled this system results in damped oscillations. We will now see what is the effect of bird predation when the parasite operates after the bird. Then, the bird only takes non-parasitized hosts and only the hosts surviving bird predation are available to the parasite. Under natural conditions this can be realized when the bird is eating larvae and the parasite infects pupae, which are inaccessible to the bird.

The steady state used in this model is given in the following diagram (P.o.I. = 2; A.o.D. = 1):



Comparing this diagram with that given on p. 366 and the steady state belonging to it (table VIII) it is easy to understand that the results wil be identical. Hence, birds operating befoie the parasite have the same effect as predators which take parasitized and non-parasitized hosts.

When the birds operate after the parasite and the parasitized hosts are inaccessible to the bird (*e.g.* when the bird takes pupae and the parasite leaves the host before the pupal stage) the model deviates on some points from the foregoing one. In diagram (P.o.I. = 2; A.o.D. = 1):



As is shown in fig. 13 the oscillations caused by a disturbance of 1.25 × S.D., are strongly damped. The calculations are partly (up to the 12th generation) given in table x.

VI. THE INTRODUCTION OF FACTORS WHICH CAN GIVE RISE TO INFESTATIONS

From the foregoing chapter it can be concluded that under certain conditions predation can damp the oscillations which are inherent in the host-parasite model of Nicholson. In part I of this study it has been shown that these conditions can be fulfilled in nature and hence, the system host-predator-parasite provides a self-regulating system.

In nature such systems can apparently be disturbed with the result that the host grows out to an infestation. Therefore, the next step in our considerations is to introduce such disturbing factors in a self-regulating system. We start from the assumption that infestations are caused by abnormal favourable weather conditions, which suddenly raise host density. The weather factors are introduced in the model by taking the I.H.D., 2 × or 4 × the steady host density. The parasite operating in the model has a limited egg supply because this probably reflects a more natural situation (*cf.* p. 357). The predator takes 68% of the parasitized and non-parasitized hosts in the steady state. Because the percentage of hosts killed by this factor is strongly increasing from low to relatively high host densities (fig. 16B) the oscillations are heavily damped.

Other properties of the model are as follows: P.o.I. = 4, A.o.D. = 1, constant parasite mortality 75%. From these data the steady densities of host and parasite can easily be calculated to be 14.08 and 0.247, resp.

The parasite has a total egg supply of 30 and, consequently, the critical host density (C.D.) amounts to this value, as well (*cf.* p. 356 and fig. 16A).

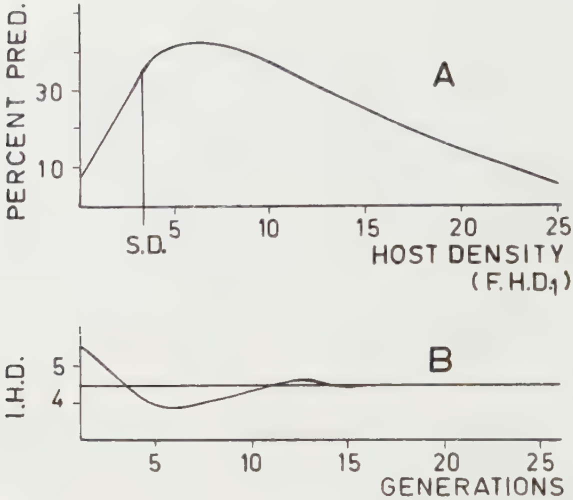


Fig. 13. Oscillations in a host-predator-parasite model. P.o.I. = 2, A.o.D. = 1. Parasite mortality 75%. Bird predation 37.5% in the steady state. *Cf.* the diagram on p. 373. Starting deviation of I.H.D. = 1.25 × S.D. The predator operates after the parasite and takes non-parasitized hosts only in quantities according to graph A. The oscillations are strongly damped. *Cf.* table x.

TABLE X

Oscillations in a host-predator-parasite model with predation density dependent corresponding to the relationship given in fig. 13. P.o.I. = 2, A.o.D. = 1. The predator operates after the parasite and takes non-parasitized hosts only. Predation 37.5% in the S.S. Parasite mortality 75%. Starting deviation of I.H.D. = 1.25 × S.D. *Cf.* fig. 13

Gen.	I.H.D.	% par.	F.H.D.$_1$	Fraction surviving the bird	F.H.D.$_2$	Density of parasitized hosts	P.D.	A.C.
S.S.	4.40	20	3.52	0.625	2.20	0.88	0.22	0.20
1	5.50	20	4.40	0.585	2.57	1.10	0.28	0.25
2	5.14	25	3.86	0.605	2.34	1.28	0.32	0.28
3	4.68	28	3.37	0.630	2.12	1.31	0.33	0.29
4	4.24	29	3.01	0.660	1.99	1.23	0.31	0.27
5	3.98	27	2.91	0.664	1.93	1.07	0.27	0.23
6	3.86	23	2.97	0.661	1.96	0.89	0.22	0.20
7	3.92	20	3.14	0.647	2.03	0.78	0.20	0.19
8	4.06	19	3.29	0.635	2.09	0.77	0.19	0.18
9	4.18	18	3.43	0.628	2.15	0.75	0.19	0.18
10	4.30	18	3.53	0.625	2.21	0.77	0.19	0.18
11	4.42	18	3.62	0.615	2.23	0.80	0.20	0.19
12	4.46	19	3.61	0.618	2.23	0.85	0.21	0.195

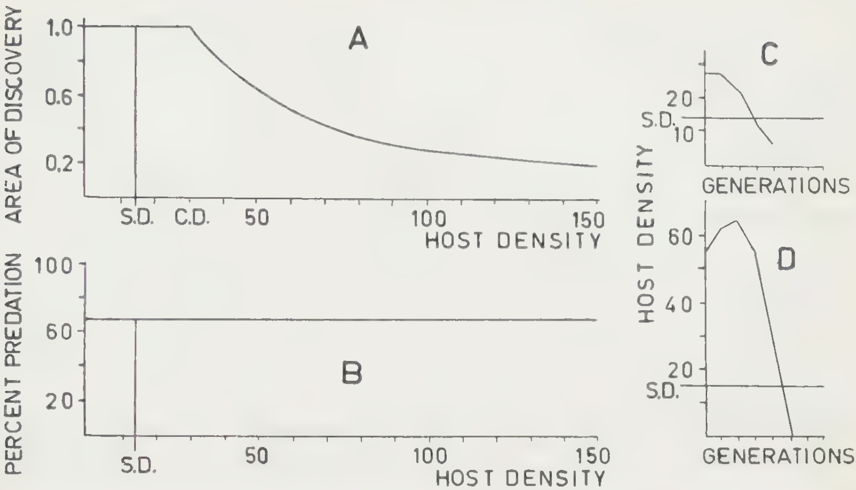


Fig. 14. Introduction of weather factors in a host-predator-parasite model by taking the I.H.D. = 2 × S.D. (graph C), and 4 × S.D. (graph D). P.o.I. = 4, A.o.D. = 1. Parasite mortality 75%. Per cent predation constant 68% (graph B). Area of discovery density dependent according to graph A. In both cases the host is unable to grow out to an infestation.

To get a clear insight in the role of parasites and predators in the model under consideration we shall first study the effect of the disturbing "weather factors" in two auxiliary models: A. The A.o.D. of the parasite density dependent and the percentage predation constant (fig. 14). B. The A.o.D. constant and the percentage predation density dependent (fig. 15).

A. The disturbance of 2 times the S.D., corresponding with a small influence of favourable weather, brings the I.H.D. to 28.16. The critical host density (C.D.) is not yet reached and the parasite prevents a further increase of host numbers. Normal Nicholson oscillations with increasing amplitudes are the result (fig. 14C).

A very favourable weather situation, however, brings the I.H.D. to 4 times the S.D., that is to a level higher than the critical density. Consequently, the activity of the parasite decreases and in the next generation the host reaches a still higher density. However, as appears from fig. 14D, the suppression of the activity of the parasite is relatively small and after two further generations the fraction of hosts parasitized is so high that the host decreases in numbers. The host is incapable of growing out to an infestation.

B. A relatively small disturbance of the steady state as shown in fig. 15C, is already levelled down in the next generation, because the percentage predation is higher than in the steady state (see fig. 15B).

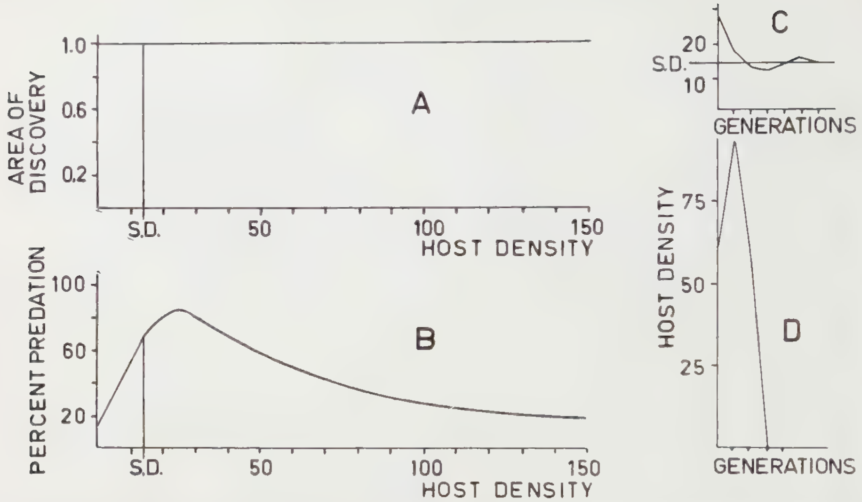


Fig. 15. Like fig. 14. Area of discovery constant. Per cent predation density dependent according to graph B. The host is unable to grow out to an infestation when taking the I.H.D. = 2 × S.D. (graph C), and = 4 × S.D. (graph D), resp.

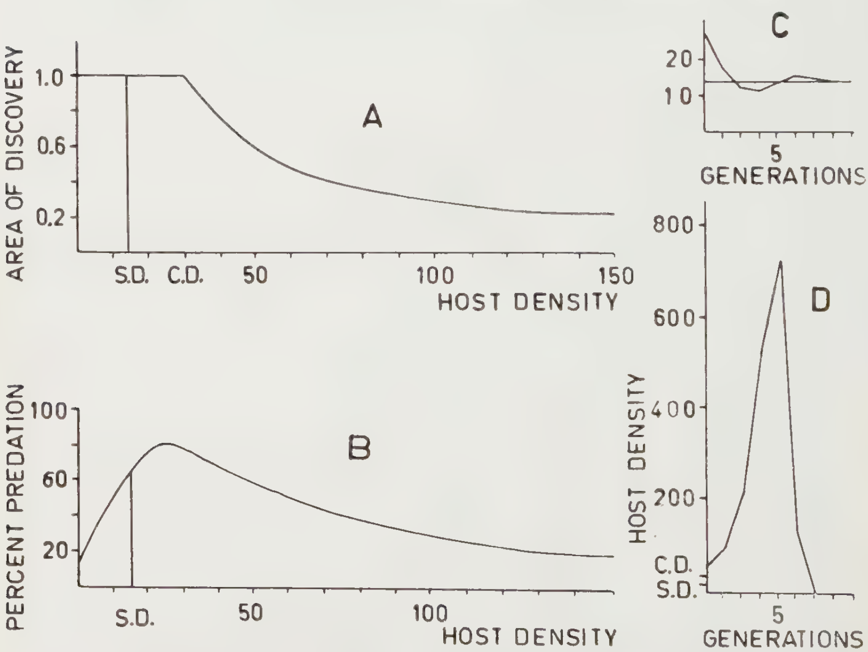


Fig. 16. Like fig. 14. Area of discovery and per cent predation density dependent according to graphs A and B, resp. Taking the I.H.D. = 2 × S.D. (graph C), the result is the same as in fig. 15C. Taking the I.H.D. = 4 × S.D. both the activity of the parasite and the per cent predation decrease and the host can grow out to an infestation (graph D).

Then, damped oscillations are the result, as has already been shown in chapter v.

When the I.H.D. is brought to 4 times the S.D., the host density is so high, that, according to fig. 15B, the percentage predation is smaller than in the steady state. Hence, in the next generation the host can reach still higher numbers. Then, the parasite has so much increased in density owing to its unlimited egg supply, that the fall of mortality due to the low predation is more than counterbalanced. The host is levelled down again, being unable to grow out to an infestation.

After the discussion of these auxiliary models we can go back to the model already noticed in the first part of this chapter (model C).

The disturbance of 2 times the steady state does not pass the critical host density. Consequently, the effect of this "weather factor" in model C is identical to that in model B (*cf.* figs. 15 and 16).

The disturbance of 4 times the S.D. brings host density to a level where the activity of the parasite is suppressed and the predator has a smaller influence than in the steady state (fig. 16). The outcome of this combined effect is a strong increase of host numbers during three successive generations, which give rise to an infestation (fig. 16D). The crash is caused by the parasite, which up to the fifth generation has increased so much in numbers that the host population is reduced to a very low density level. The predator, on the other hand, can in no way contribute to the occurrence of the crash, because its numbers are constant (*cf.* p. 363) and, consequently, the percentage predation can not increase.

Now, it will be evident that in theory the role of birds in the regulating mechanism of caterpillars seems to be contradictory. On the one side they have a stabilizing influence by damping the oscillations. On the other hand they can contribute to the occurrence of an infestation because they eliminate a decreasing fraction of the hosts at higher densities.

VII. SUMMARY

The main objections which have been levelled against the theory on host-parasite interactions of Nicholson (1933) are concerned with the constant power of increase of the host, the invariable searching activity of the parasite and the ever-increasing amplitudes of the oscillations in the arithmetic models. Moreover, under natural conditions the host-parasite system will be more complex and include, *e.g.* mortality factors of the host and the parasite.

In this paper the initial assumptions of Nicholson have been reconciled with the experience of fieldworkers, to see whether this leads to self-

regulating systems. The effect of several fluctuating and density dependent factors has been studied arithmatically, *e.g.* a fluctuating weather factor (chapter II), density dependent power of increase of the host and searching activity of the parasite (chapters III and IV), density dependent mortality of the parasite and the host (chapter V).

The conclusions reached can be summarized as follows:

1. Fluctuating weather factors have no major effect on the general course of the oscillations. The amplitudes still increase in size. The phase of the oscillations may be shifted some generations due to the random succession of favourable and unfavourable years (fig. 3).

2. A strongly density dependent reproduction (P.o.I.) of the host results in damped oscillations (fig. 4). It is unlikely, however, that this mechanism of damping operates in nature. For, it has never been observed that the fecundity falls greatly at density levels which are slightly above the mean density.

3. NICHOLSON assumes the fecundity of the parasite to be unlimited, and the searching activity independent of host and parasite density. From this can be derived that the area of discovery is constant. It is argued in chapter IV of this paper that these assumptions are unlikely to occur under natural conditions. The egg supply will be limited and, moreover, each egg deposition may have a temporarily inhibitive influence on the activity of the parasite. Then, the area of discovery decreases with increasing host density (fig. 5). When a parasite with such properties is introduced in a host–parasite model, the phenomenon of the increasing amplitudes is strengthened (fig. 6).

4. In chapter V a host-parasite model of NICHOLSON is combined with empirically determined bird predation. A long term investigation of the influence of titmice on caterpillar populations in pine woods (see part I, this volume) revealed that in the lower range of densities of single prey species the percentage predation strongly increased with increasing density. This effect is due to the development of a preference by the titmice for prey species at intermediate densities (fig. 8).

When the birds eliminate a considerable part of the population at intermediate densities of the prey (more than 25%) this density dependent predation has a damping effect on the oscillations of a host–parasite model (fig. 10). Consequently, under certain conditions, the system host–predator-parasite is self-regulating.

5. Introduction of (weather) factors which suddenly raise host density to about four times the steady density can give rise to infestations of the host, particularly in systems the oscillations of which are damped by bird predation. This last effect is due to the fact that the percentage predation strongly falls in the range of higher host densities because the density of the birds remains constant, whereas the prey

population increases. Moreover, the birds try to vary their diet and avoid species which are very numerous (figs. 8 and 16).

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PROTECTION AGAINST BIRDS AND PARASITES IN SOME SPECIES OF TENTHREDINID LARVAE

by

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CONTENTS

I. INTRODUCTION

L. Tinbergen (1949, 1959) has studied, over a number of years, the relative densities of various species of larvae living on pine (*Pinus sylvestris* L.) in a wood near Hulshorst, Netherlands. Simultaneously he determined the composition of the food wild tits in this wood collected for their young. One fact resulting from this study is that, whereas in some prey species a considerable part of the population was eaten by birds, the populations of others, e.g., of sawfly larvae belonging to the genera *Diprion* and *Neodiprion* (Hymenoptera: Diprionidae), were scarcely affected by predation. At Prof. Tinbergen's suggestion I undertook an investigation on the reason why these larvae run so little risk from birds. As protection by cryptic or by aposematic characters (cf. Cott, 1957) seemed the most likely explanation, I studied the conspicuousness of the larvae on pine needles (chapter III) and of their attractiveness as prey to birds (chapter IV). Chapter II gives experiments and observations on meetings between the larvae studied and birds; in addition, encounters with parasites have been investigated. A final discussion on the nature of the protection against predation by birds follows in chapter V.

I have studied five congeneric species (*Diprion virens* Kl. (syn. *Gilpinia virens* Kl.), *D. frutetorum* F. (syn. *Gilpinia frutetorum* F.), *D. nemoralis* Ensl., *D. simile* Htg., *D. pini* L.) and the closely related *Neodiprion sertifer* Geoffr. (syn. *Diprion sertifer* Geoffr.). *Diprion pallipes* Fall. and *D. pallidum* Kl., also occurring in the Netherlands, have not been studied.

For specific descriptions and life history of the species mentioned I refer to Escherich (1942) and De Fluiter (1932). Moreover, our special subject asks for descriptions of the following behaviour patterns.

When disturbed by an enemy, either a bird or a parasite, the larvae perform more or less conspicuous displays. Three types can be distinguished:

U-bend. With a rapid jerk the larva bends its head and thoracic segments backwards, often also raising the end of the abdomen, until

the rostral and caudal parts make an angle of about 90° with the rest of the body (Plate 1, 1). Moreover, a drop of a whitish liquid appears on the mouth parts (Plate 1, 2). As a rule, this mouth drop is withdrawn after some ten seconds; the convulsively bent attitude itself may last for 10 minutes or even more. The form of this activity is almost the same in the six species studied. Intraspecific variations occur in the number of abdominal segments lifted (maximal 4) and in the angle over which the frontal part is raised.

Jerking. Like in the U-bend, the head, the thoracic and in this case also one or more of the rostral segments are lifted from the substratum until they are about at right angles to the attached rest of the abdomen; then they are immediately swept back again. The last abdominal segments remain attached to the needle. The jerking is often repeated rhythmically at a high rate. As an illustration, fig. 1 gives the results of a series of 42 experiments in which a short lasting stimulus (swinging a pencil 2 cm away from the colony) was presented to a *sertifer* colony. The figure shows that in the first 15 second period after stimulation jerking was more than 20 times as frequent as in the preceding control period in which no artificial stimulation was given; these jerkings were due to insects which had passed. In the second 15 second period a rapid decrease followed.

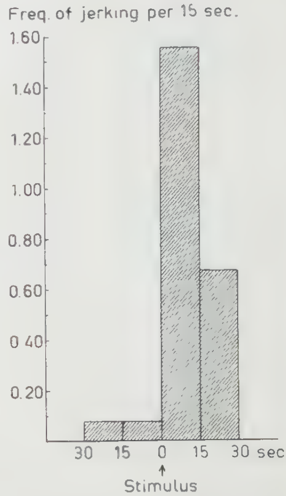

Fig. 1. Rate of jerking in a *sertifer* colony before and after a visual stimulus.

This display which has already been mentioned by Iltis (1930), Escherich (1942) – who called it "schnippen" – and Husson (1952), is restricted to the gregarious species *sertifer* and *pini*. However, these show some specific differences. *Sertifer* performs jerking at a higher rate than *pini*, it also bends farther backwards than the latter. If in a *sertifer* colony more than one larva have been activated, they as a rule jerk simultaneously, whereas in a *pini* colony the jerking of different members is never synchronized. Characteristic for *pini* is that, during bending backwards, the thoracic legs are detached successively; the tarsus of the

PLATE I

Fig. 1. *Neodiprion sertifer* Geoffr. Larvae in U-bend. (Photograph by Mr. K. P. van Kempen.)

Fig. 2. *Diprion pini* L. Responding larva with a mouth drop. (Photograph by "Centrale Fotodienst", University Groningen.)

PLATE I

1

2

1

PLATE II

2

second and particularly of the third pair hold on for such a long time that the needle is slightly bent. Consequently, when a moment later also these legs are detached, the needle springs back with a visible quake.

Stretching. The thoracic legs and the first two or three pairs of abdominal legs are detached from the substratum; the fore part of the body is not swept backwards (as in jerking), but on the contrary, the larva stretches it and turns towards the source of the stimulus. This orientation is continuously corrected when the stimulating object moves. Like in the U-bend, the larva produces a mouth drop which, while stretching, it tries to smear on the stimulating object (Plate II, 1).

Like jerking, this activity occurs only in the gregarious species (*pini* and *sertifer*). The form of the activity is similar in both species.

Two completely different kinds of function have been suggested for these displays. RATZEBURG (quoted by ESCHERICH, 1942, p. 67) assumes a physiological effect of jerking in facilitating digestion. However, this seems rather unlikely, because, when feeding undisturbed, the larvae never show jerking. It is more generally held that the displays serve to protect the larvae against enemies. This paper is an attempt to deal critically with only the latter aspect of the displays.

With regard to a protective function we can discern the following possibilities:

1. The displays might afford an active defence.
   a. They might appeal to a disposition in birds and parasites to become startled by sudden movements, and thus act without being actually dangerous to an enemy.
   b. The displays might mean a real danger for the enemies in causing harm to small attackers, such as parasites.
2. The displays might be part of an aposematic advertisement, based on an association of conspicuousness and unattractiveness.
3. The displays might have the effect of directing an attack to a part of the animal with special defensive properties.

Acknowledgements: I am deeply indebted to the late Prof. Dr. L. TINBERGEN for drawing my attention to the problem and for help, criticism and encouragement. I have often benefited from his extensive theoretical knowledge and from his admirable experience with animals in the field. This study is part of the ecological research in the field station

PLATE II

Fig. 1. *Diprion pini* L. Larvae stretching (×) towards a swinging twig (+). (Photograph by Mr. K. P. VAN KEMPEN.)

Fig. 2. *Diprion pini* L. Colony in a dense cluster. (Photograph by Mr. K. P. VAN KEMPEN.)

of the Zoological Laboratory of Groningen University at Hulshorst. I wish to express my great appreciation for Prof. TINBERGEN's friendly and effective leading of all the students who participated in his Hulshorst work.

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I am further indebted to Mr. P. KUYTEN for his assistance in collecting larvae for the experiments and for identifying some of the parasites, to Mr. T. HEIKENS for making the drawings and to Mr. K. P. VAN KEMPEN for making some of the photographs.

II. THREAT AS A FACTOR IN THE PROTECTIVE FUNCTION OF THE DISPLAYS

1. INTIMIDATION OF BIRDS BY THE DISPLAYS

Experiments have been designed to investigate two questions at the same time: viz. (1) can an approaching insectivorous bird release the above mentioned displays in the larvae, and (2) are these displays able to evoke fright responses in the birds.

A. Material and technique

Test birds were two captive Chaffinches (*Fringilla coelebs* L.) and a captive Great Tit (*Parus major* L.). Their cages measured 158 × 78 × 78 cm. Finch R_0 was used in experiment 1, 2 and 9, Finch R_2 in exp. 10 and 11, the Tit in exp. 3, 4, 5, 6, 7 and 8. Some additional observations were made on wild birds visiting feeding trays; for technical details of these experiments I refer to chapter IV.

The Great Tit was reared in captivity from just before the fledgling stage. At the beginning of the experiments it was acquainted with anaesthetized *Diprion* larvae, but not with living specimens. The Finches had been caught four months before they were used in the experiments. During captivity they had not had any experience with the larvae.

Encounters between birds and larvae were arranged as follows. The larvae were presented on a pine twig of about 30 cm length. The twig was laid on the bottom of a cage or on a feeding table for wild birds, very cautiously to prevent disturbance of the larvae. The captive birds were

accustomed to twigs in the cage; their staple food was not removed during the tests.

As a fright response I consider a quick hopping or flitting away after the bird had distinctly taken notice of the larvae. During such a flight the wings drooped a little, neck, body and legs were stretched, the feathering was sleek. Calls were never evoked in the birds by the activity of the larvae. I made sure that the birds were not pathologically susceptible for sudden movements in their vicinity. They showed normal behaviour in all respects, as described for tits by HINDE (1952) and for Chaffinches by MARLER (1956).

B. Experiments

Exp. 1. The rate of jerking in a *sertifer* colony when inside a cage containing a Finch, was compared with its rate of jerking in a preceding control period in which the colony was outside the cage without the presence of a predator. Care was taken not to disturb the colony when bringing it into the cage. The Finch approached the colony but did not peck at it. Nevertheless, the rate of jerking — 0.7/minute with a standard deviation of 0.09 (averaged over 13 minutes) during the control—increased to 8.3/minute $\pm$ 2.5 (averaged over 14 minutes). This difference, obviously caused by the presence of the bird, is significant ($t = 5.1$, $P < 0.01$).

Exp. 2. A *sertifer* colony of about 20 larvae was brought into the cage of a Finch. During the next 50 minutes the Finch hopped to the colony 27 times but did not peck at it. Twenty of these approaches released jerking. Here the sudden jerks resulted in clear escape responses of the Finch. In the other 7 cases the larvae did not respond and the Finch hopped away quietly.

A series of similar experiments was undertaken with a Great Tit.

Exp. 3. During 60 minutes a small *pini* colony of seven larvae was placed in the cage of the Tit. The Tit approached the twig 13 times; apparently, its interest for the colony was rather low. The first 6 visits—until the 26th minute—each time released stretching in the colony to which the Tit reacted with flight. The larvae did not respond to the last 7 approaches. Then the Tit attacked 3 larvae but dropped them again. Once the Tit pecked at the twig; although the larvae responded with U-bends, the Tit did not appear startled; it even attacked a responding larva, again without eating it.

Exp. 4. Nine months after exp. 3, a *sertifer* colony of about 20 larvae was placed in the cage of the Tit. During the 60 minutes of the experiment the bird visited the twig 11 times, showing as little interest in the colony as in exp. 3. The larvae reacted to 9 of these visits: 1 time with jerking, 5 times with stretching and 3 times with U-bend. Two visits did not release any response. The Tit was frightened by the jerking, not by stretching and U-bends. It even attacked a stretching larva (already in the 40th second of the experiment) and a larva in U-bend, without eating them, however. No other attacks took place.

Exp. 5. Twelve days after exp. 4 during 30 minutes a *sertifer* colony of about 20 larvae was presented to the Tit. The bird approached 19 times. In 16 of these the larvae responded with stretching and jerking, in 3 no reactions were released. The Tit showed no overt fleeing responses; it even attacked one stretching larva in the second minute of the experiment but did not eat it. No other attacks were tried, neither on the displaying nor on the non-responding larvae.

Exp. 6. A pine twig with one *nemoralis* and one *simile* larva was put into the Tit's

cage. The first time the Tit approached, the two larvae responded with a U-bend, but this did not frighten the bird. It even pecked at the mouth drop of both larvae. After bill wiping, the Tit attacked *simile* (in the fourth minute) and ate it. The *nemoralis* larva responded again with the U-bend to the next approach. The bird, without showing signs of fright, attacked (in the ninth minute) and swallowed the larva.

Exp. 7. Four *virens* larvae were placed on a pine twig in the cage of the Tit. The larvae did not react to the bird, which quietly consumed one larva in the 12th second and the others in the 2nd, 7th and 9th minute.

Exp. 8. Three *frutetorum* larvae on a pine twig were brought into the cage of the Tit. The larvae did not respond when the Tit approached in the second minute. The bird only just pecked at larva A but then attacked and swallowed B. Larva A reacted to eight further approaches with the U-bend, larva C remained quiet. But neither of the two was taken, until at the tenth approach when A, again in the U-bend, was attacked and consumed (in the 36th minute). In the next minute the non-displaying larva C was eaten.

I know from many experiments (which will be produced later on) that the gregarious larvae do have prey value to Chaffinches and Great Tits. Nevertheless, in exp. 1, 2, 3, 4 and 5 attacks were rare. Therefore, the question arises whether a possible frightening effect of the colony (either in rest or displaying) might be responsible for keeping a finch or a tit from attacking one of its members. If this effect would be found, it would be of interest to know whether it remains constant or changes in an individual after repeated experience with the colony. I, therefore, designed the exp. 9, 10 and 11.

Exp. 9. Test a: An anaesthetized mealworm (*Tenebrio molitor* L.), lying on a pine twig, was offered to a Finch. The Finch took this mealworm without any hesitation. Test b 1: an anaesthetized mealworm was presented in the same situation, but on a twig that also carried a *sertifer* colony, 2 cm away from the mealworm. This situation evidently caused a conflict in the Finch. It approached cautiously, continuously watching the colony. The larvae did not react and the Finch quietly took the mealworm. Test b 2: The experimental set-up was as in the preceding test. However, this time the larvae jerked when the Finch approached. The bird was evidently frightened and flitted away from the twig. A second and a third approach also ended in flight. In the fourth approach the larvae responded again, but then the Finch pecked the mealworm hastily from the twig and hopped away quickly.

Exp. 10. Using another Finch as a test bird and a fresh *sertifer* colony of about 20 larvae, I repeated exp. 9 in a more extensive form. Fig. 2 shows on the abcissa the order in which tests a and b (presentation of a mealworm without or with a *sertifer* colony respectively) were made and on the ordinate the time between presentation of the mealworm and attack of the bird. The pauses between two successive tests were about five minutes. Although in this experiment the larvae did not respond to the Finch when it approached, the bird was strongly frightened in the first three b-tests. Only in the very first of these I could score an attack (in the 14th minute); then the Finch flitted away with its prey from the colony. From the behaviour of the bird it was clear that already the mere presence of the colony caused an increase of the tendency to flee: the Finch hopped quietly to the twig in the control tests, it was very alert and agitated in the b-tests, hopping to and fro near the twig or looking at the

colony with its neck and legs stretched. The bird's anxiety was so persistent that two tests had to be stopped when the mealworm was not yet taken by the bird, despite a relatively long time of presentation. The tendency to flee waned in the course of each experiment as well as with every consecutive experiment. In the last b-tests the Finch attacked without delay.

Exp. 11. The next day I repeated exp. 10 with the same Finch and another, similar *sertifer* colony (fig. 3). Except for the first one the Finch took the mealworms

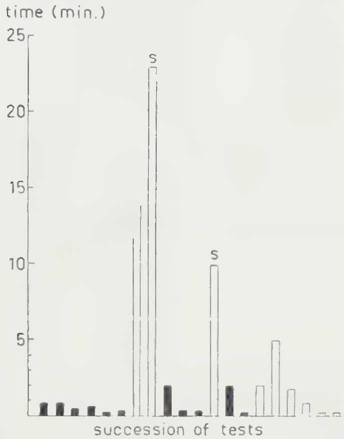


Fig. 2. Results of Exp. 10; time passed between presentation of a mealworm and the attack of the Chaffinch. Black columns: in absence of a *sertifer* colony. White columns: in presence of a *sertifer* colony. The tests marked with s were stopped when the mealworm, in presence of a colony, was not attacked despite a relatively long time of presentation.

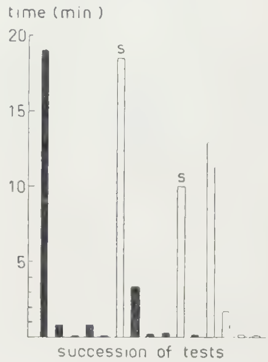


Fig. 3. Results of Exp. 11; legend as in fig. 2.

quickly in the a-tests. In the b-tests the Finch was evidently startled by the now jerking larvae, again even to such an extent that the first 2 tests were stopped before the mealworm had been attacked. In the third b-test the bird took the bait in the 13th minute; in the last three b-tests the delay decreased rapidly.

In an attempt to check whether the results can be applied to birds in the field, experiments with wild birds were undertaken.

Exp. 12. A pine twig with a *sertifer* colony was laid on a feeding table in the territory of a Great Tit breeding pair. The female visited the table four times. As far as I could observe, at each visit it pecked two larvae from the twig and carried these to the young. The colony responded feebly; the Tit behaved quietly.

Exp. 13. On the next day a similar experiment was carried out. After each of its first 2 visits the female carried 2 larvae to the young. At the third visit the larvae were left undisturbed. In the 4 next visits 7 larvae were taken. Again the responses of the larvae were feeble while the Tit behaved quietly.

Exp. 14. On a feeding table in the territory of another breeding pair a pine twig was placed carrying a *sertifer* colony to which, near its periphery, a mealworm had

been added. The male Tit took the mealworm at its first visit without being visibly influenced by the jerking colony. During the second visit, the Tit hopped over the colony when searching for food on the table. Again the renewed jerking did not startle it, although it clearly observed the vigorously moving larvae. However, the bird did not attack any of the larvae. At the next visit to the table the Tit again searched for food, its head sometimes close to the then rather feebly responding colony. No larvae were touched.

Once I observed the same Tit as in exp. 12 and 13, when it was feeding on a *sertifer* colony in a pine tree. In 6 rapidly succeeding visits it carried an unknown number of larvae to its young. I could not observe the colony itself.

C. Discussion

Although the experiments are not homogeneous in design, it is worthwhile to summarize the results tentatively, as far as possible. In exp. 2 to 8 the approach of the bird released a display 64 times (jerking, stretching or U-bend). In 42 cases the larvae did not respond. The bird was seen to be frightened after jerking in 21 out of 29 cases, after stretching in 6 out of 19 cases and after the U-bend in 0 out of 16 cases. In 6 cases a displaying larva was picked up (2 when stretching, 4 when in U-bend), in 9 cases a non-displaying larva.

Thus, neither did the larvae react in all cases, nor were the birds always driven off by the displays. However, as is generally accepted, protection from predators can be far from infallible and still have a considerable selective advantage to a species. It is evident that the design and the small number of my experiments prelude any attempt to estimate the quantitative value of this advantage.

Whereas the gregarious larvae can frighten an avian disturber by jerking or stretching, or even merely by being aggregated, the U-bend of the solitary larvae could not be shown to have a frightening effect. Such an effect might have been found after further experimentation. When displayed by a colony, the U-bend may be expected to enhance the already existing intimidating effect of mere aggregation. Another function of this display will be mentioned in chapter V.

It is also remarkable that, whereas all solitary larvae were consumed, in the experiments with the captive birds none of the gregarious larvae has been eaten. This can be explained by the finding in chapter IV that the solitary larvae are more highly appreciated.

However, a protective effect of the displays was demonstrated with caged birds only, not with the wild ones. One wonders how this disturbing difference has to be explained. It seems very unlikely that the difference between wild and captive birds is really qualitative. Unfortunately, because of a shortage of larvae when wild birds were willing to visit the feeding tables, it was impossible to extend the number of experiments with wild animals. Although my observations give as yet

no possibility for a satisfactory explanation of the discrepancies found, I strongly believe that if further experiments would still show significant differences between wild and captive birds, these differences would prove to be of a quantitative nature only.

One might ask how long and in what way the frightening effect exerts its influence. There is reason to assume that the intimidation induced causes an avoidance for a certain time. As MARLER (l.c.) has pointed out, avoidance of unfamiliar objects which involve some element of danger, is a rule in small birds, although this is counteracted to some degree by exploratory behaviour, i.e. by a tendency to examine all strange objects. The results of these two opposing tendencies, according to MARLER, is examination of strange objects at a safe distance. I do not know the duration of this avoidance behaviour. It may be that, as long as alternative food is abundantly available, a bird does not learn to overcome its resistance against attacking gregarious larvae. At times of food shortage, however, the intimidating effect will surely afford but a part-time protection. For then attacks on the gregarious larvae are bound to come; and these will lead inevitably to a more or less rapid break-down of the frightening effect. Here I may refer to exp. 9, 10 and 11, which clearly show waning of the frightening effect in the birds. In this context I may also refer to BLEST (1957) who found rapid habituation in small birds to the displays of eyespot bearing butterflies; after a month's rest the recovery of the responsiveness was only slight.

Fading away of the intimidating effect —whatever its nature may be: learning, adaptation or some other physiological process— does not yet imply defenselessness in the larvae. In my observations only one Tit preyed upon the gregarious larvae; other birds, although behaving very quietly near a colony, let these larvae undisturbed or, when they attacked, did not eat them. Apparently, the colonial larvae were safe from attacks because of being unattractive. This suggestion will be confirmed in chapter IV and V.

We can envisage what may happen to birds that come into contact with sawfly larvae. In the beginning of each summer period, part of the birds, if not all, will be inexperienced. The displays of the gregarious larvae then may act upon an already present susceptibility to uncommon and sudden visual stimuli in these birds. The birds are frightened and avoidance results. When encounters with the gregarious larvae accumulate, waning of the avoidance responses may be expected. However, if this leads to actual attacks, the birds will discover the bad taste of the larvae. It seems likely that from then on the displays no longer work through frightening the birds but through reminding them of disagreeable properties. This aposematic aspect of the displays will be discussed in chapter V.

It may be asked what is the relative importance of the frightening and the aposematic effect in the total protective value of the larval displays. Observations of TINBERGEN prove that the larvae are very effectively protected against experienced birds as well. This suggests that for the problem just mentioned a knowledge of the duration of the intimidation phase is critical. As already said, I cannot present suitable information on this point.

2. INTIMIDATION OF PARASITES BY THE DISPLAYS

In view of the very important ecological relationships between larvae and their parasites I have also paid attention to the reaction of some parasites to the displays of the sawfly larvae. As in the preceding section, I consider the attacker to have been frightened when it evidently responded to the displays by retreating or flying off.

A. Material and technique

On an open space in a pine wood five colonies of *sertifer* were placed each on one low pine tree. Moreover, about 30 solitary larvae (*frutetorum*, *virens* and *nemoralis*) were distributed over 10 cut pine twigs (one species per twig) which were placed in pots filled with water. This set-up could be surveyed easily.

ESCHERICH (1942) gives a survey of the most important parasites of *Diprion* larvae. Regarding the hymenopterous parasites, I have confined my observations to the genus *Exenterus* (Hymenoptera, Ichneumonidae), well-known as specific parasites of *Diprion* larvae. The observations were carried out in the end of June and in the first week of July. The *Exenterus* specimens were easily recognizable as to their generic characters; their size, the black and yellow bands and especially their behaviour provided sufficient certainty. Three specimens I have caught for classification. According to Mr. P. KUYTEN two were individuals of E. *marginatorius* F. and one of *E. cingulatorius* Holm. Further catches were not attempted, as it was undesirable to interfere with the observations. As specific differences could not be determined in free animals, I do not know to which species of Exenterus each of the observations refers. For my purpose this is permitted, particularly as I have not noticed any behavioural difference between the individuals.

Mid-July some colonies of *pini* were used for observation. Then I could observe two species of Tachinids (Diptera): *Sturmia inconspicua* Meig. (det. Mr. P. KUYTEN) and a still unidentified species.

Exenterus and the Tachinids are ectoparasites. An egg deposited on the skin of a host is visible with the naked eye as a white spot. This

made an immediate statement on the success of the parasitation trials possible.

B. Observations on Exenterus *spec.*

An *Exenterus* specimen, after some flying round the twigs, alights on a branch or a needle and begins to walk. While walking it moves its antennae continuously, probably in search of hosts. Sometimes it passes a solitary larva at a distance of about half a centimeter, apparently without perceiving it, although in other cases a motionless solitary larva is perceived when the antennae of the parasite are still about 2 cm from it.

When perceiving a larva, the parasite suddenly changes its behaviour. It begins a very slow stalking (like the american *Exenterus platypes* Cushman; GREEN and DE FREITAS, 1955), keeping the antennae motionless and directed towards the host. When the antennae have almost reached the larva, they are very slowly moved over it, generally without touching it visibly. This "groping" movement of the antennae presumably serves as a test. Sometimes the wasp walks off after groping, without being disturbed by a response of the larva; in other cases, immediately after groping, it suddenly jumps upon the larva, oviposits and flies off again. Approximately one second suffices for ovipositing. The attacked larva tries to push away the parasite by an upward jerking of head and thoracic segments. A timely response makes oviposition impossible.

Surprisingly the eggs are always laid upon the dorsal or dorsolateral side of one of the thoracic segments, often upon the first of these. It becomes understandable by the fact that *Exenterus* always jumps upon the larva with its head directed to the caudal end of its victim (fig. 4). This suggests that testing with the antennae also serves orientation.

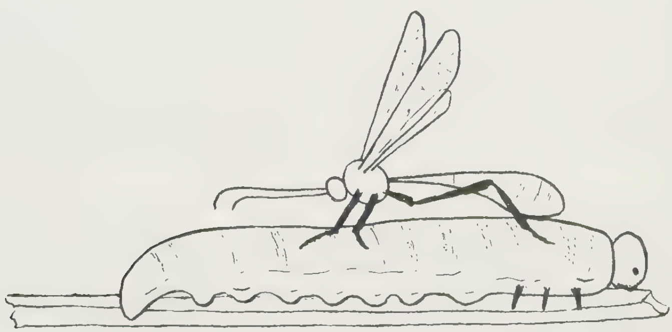


Fig. 4. The head of a parasitizing *Exenterus* is always directed to the abdomen of the larva.

I have observed successful ovipositions on *virens* and *frutetorum*. For some unknown reason — perhaps because of host selection — the twigs with *nemoralis* larvae were not visited by the parasites. The wasp also succeeded in stalking *sertifer* colonies. Sometimes a parasite walked

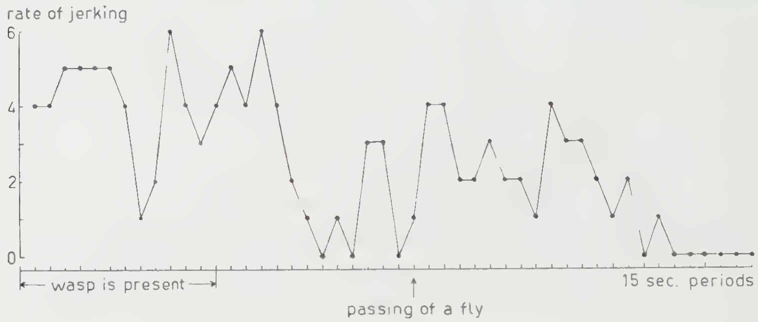


Fig. 5.

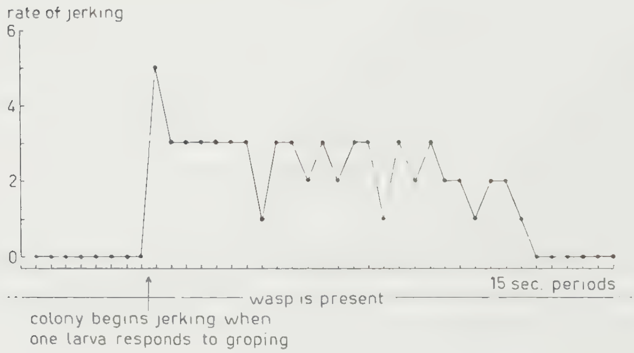


Fig. 6.

Jerking in *sertifer* colonies as a response to the presence of an *Exenterus* specimen. Plotted are the numbers of jerks (ordinate) in successive 15 seconds periods (abcissa). – Fig. 5. The colony is jerking at a high rate when the parasite is present. The jerking decreases after the wasp's departure. It re-appears for a short time when a fly passes. – Fig. 6. At the beginning the colony is quiet in spite of the wasp's presence. When, however, a larva responds to groping, the colony begin an intense jerking that decreases gradually.

around, stalking and groping, in or near a colony, during as much as an hour without eliciting any response in the larvae. In other cases, however, a parasite evoked vivid responses —jerking and stretching— by moving too rapidly or by touching a larva with a leg or an antenna. These displays always released flight in the parasite. Sometimes a larva that was not directly approached or threatened responded first.

Such cases prove that gregariousness results in a higher watchfulness.

Fig. 5 and 6 exemplify the disturbance of a *sertifer* colony by the presence of an *Exenterus* specimen. The effect is also illustrated by the following record.

A wasp remains near a *sertifer* colony during 64 minutes. The parasite shows almost constant stalking and, as far as possible, groping behaviour. In this period the colony jerks 220 times or, on the average it performs 3.44 jerks per minute. After the wasp has disappeared the rate of jerking immediately falls back to 0.09 jerks per minute.

That a parasite can disturb a colony merely by moving too rapidly, as asserted above, is substantiated by the following experiments. A 5 cm long empty glass tube, closed with a stop of cotton-wool, was held by means of a piece of wire at 5 cm from a *sertifer* colony. I recorded the jerking frequency per period of 15 seconds over a certain time. Then, all other things remaining equal, an active fly was put into the tube. The walking fly induced a significant increase of jerking from 0.38 (with a standard deviation of $\pm$ 0.48) to 2.08 $\pm$ 1.07 (number of unit-periods = 24; $t = 27.4$, $P < 0.01$). A second experiment also showed an increase, from 0.64 $\pm$ 0.88 to 2.90 $\pm$ 1.20 (number of unit-periods = 22; $t = 6.8$, $P < 0.01$).

Quantitatively the observations have been summarized in table 1.

C. *Observations on Tachinidae*

I made several observations on *Sturmia inconspicua* when it was attacking *pini*. Generally, the fly alighted on a needle 4–5 cm away from a colony. There, facing the colony, it stayed motionless for some minutes. The arrivals always released an intense stretching and jerking in the colony. In several cases the larvae stretched themselves towards the fly, with thick drops on their mouth parts. However, the fly was never within reach. Because the fly did not move, the activity of the larvae decreased gradually. Then, suddenly, the parasite flew, very rapidly, towards one of the larvae and, within the same second, returned to alight on a new starting-point (Escherich uses the term "blitzartig" in this context). This short moment sufficed to deposit an egg on the attacked larva.

One morning, with warm and sunny weather, the fly had an imposing success: all its trials to parasitize succeeded. In one and a half hour I recorded in three *pini* colonies 18 ovipositions. The responses of the colonies had, despite their high intensity, no effect at all since they were only exhibited after the fly had returned to its starting-point. However, later observations gave me the impression that the success of the parasite is strongly influenced by weather conditions.

A very different technique I once observed in another Tachinid, distinctly smaller than *Sturmia*. Like *Exenterus*, it stalked towards a colony, approaching one of the larvae very closely without causing disturbance. Then it bent its abdomen forwards between its legs, meanwhile protruding a proboscis shaped ovipositor. The fly, manoeuvering very cautiously, succeeded in bringing its ovipositor repetitively in touch with the larva; after the third contact a small, cream-coloured egg was visible upon the larva which had not shown any response. The fly has not been identified because, unfortunately, I observed it only once. Below it is referred to as Tachinid X.

Remarkably, WEBBER (1932) describes a similar stealthy parasitizing technique for *Sturmia inconspicua*. But, apparently, his Tachinid cannot be identical with the fly which dives rapidly to its host and has been called *Sturmia inconspicua* by ESCHERICH and by me. Therefore, I want to stress that *inconspicua* in my observations answered in all its morphological details the description of this fly by SACHTLEBEN (1942).

D. *Conclusions and discussion*

a. *The effect of the responses on the success of the parasite.*

Stalking in *Exenterus* individuals was never broken off by a response of a *virens* or *frutetorum* larva. The responsiveness of *sertifer*, on the contrary, was shown to interfere often with stalking (in 33 % of the total; table 1). The difference between the results on (*virens* + *frutetorum*) and *sertifer* is significant ($X^2 = 8.58$, $P < 0.01$).

Groping of *Exenterus* has been disturbed by *frutetorum* and by *sertifer*, not by *virens*.

It can be inferred from the table that the proportion of walking off

TABLE I

The effect of different elements of the parasitizing behaviour of *Exenterus* spec.

Host	Frequency of occurrence and effect of the elements							
	Stalking		Groping				Oviposition	
	no response released	response released	no response released	response released	Walking off	Jumping	no egg laid	egg laid
virens	17	0	17	0	4	13	7[1])	6
frutetorum	6	0	4	2[2])	1	3		3
sertifer	48	24[3])	44	4[4])	42	2	2[1])	0

[1] Failures were caused by a timely response of the larva. [2] Larvae were touched by an antenna; their bending upwards made the wasp flee. [3] The parasite released jerking or stretching by walking too rapidly; in 21 cases the displays resulted in a retreat of the wasp, in 3 cases stretching made the parasite drop off the needle and fly away. [4] Touched by the antennae of the groping parasite, the larvae responded with jerking and thereby caused the wasp to retreat.

in relation to jumping was much higher in *sertifer* than in (*virens* + *frutetorum*) ($X^2 = 32.8$, $P < 0.01$). Perhaps this difference is due to host preference (cf. FINLAYSON, 1950). Another cause might be selection of a special instar. This is supported by a statement of SCHEIDTER (1934, quoted by ESCHERICH, l.c.) that *Exenterus marginatorius* only parasitizes "Einspinnstadia" of *Diprion* larvae. SCHÖNWIESE (1935) reports the same for *Exenterus cingulatorius*. However, such a rigid instar selection does not hold for my observations, since larvae in the final instar were also found parasitized. Pilot experiments in which anaesthetized larvae in the final instar were used gave me the impression that *Exenterus* will lay an egg only on a larva that is quite motionless during the parasite's approach. It might be, therefore, that feeding larvae are in general too mobile for these parasites.

Table 1 shows that 44 out of 72 approaches of *Exenterus* to a *sertifer* larva have led to successful groping. The fact that part of the trials failed implies that now the wasp must spend more time stalking and groping than when the larvae would be insensitive to the stimuli from an active parasite. The reactions of the larvae thus lead to a decrease of the rate of oviposition; the effect will be much smaller of course in *virens* and *frutetorum* than in *sertifer*.

This depressing effect must always be disadvantageous to the parasite. If the number of eggs deposited per female would be determined by the life span of the wasp, each lowering of the rate of successful attacks would necessarily result in a reduction of the total number of ovipositions per female. If, alternatively, the wasp would live at least as long as necessary to dispose of all its eggs, ovipositions would be spaced out over a longer period. But the wasps are bound to suffer a certain mortality rate. Each lengthening of the period in which the parasite is active implies, therefore, a proportional increase of the number of victims in the wasp population.

Thus it may be assumed that in *sertifer* the responsiveness of the larvae causes a shift in the ratio between the reproductive rates of the sawfly and *Exenterus* in favour of the former. Therefore, a rapid increase of a *sertifer* population will be less effectively counteracted by *Exenterus*. Now *Exenterus* is not the sole parasite on *sertifer* but it is an important one (see ESCHERICH). SCHÖNWIESE (1935) even reports that during a pest of *sertifer Exenterus* parasitized 64 % of the sawfly larvae. It is clear that in such cases the critical check of the pest by the total activity of all parasites will be reached at a higher density level of the sawfly population than when defensive responses of the larvae towards a main parasite would be lacking.

In 21 cases *sertifer* larvae caused a retreat of a stalking wasp, in 3 cases their stretching made the parasite drop itself from the needle and fly off.

These three observations are highly interesting. Since the wasp had penetrated far into the colony, it was within reach of the mouth drop of some stretching larvae. These could have smeared the wasp with their sticky secretion if the parasite had not fled in haste. Experimentally I could prove that touching insects such as flies with one mouth drop invalidates them completely. Therefore, it does not seem impossible that occasionally an *Exenterus* specimen is eliminated by being smeared by *sertifer* or *pini* larvae.

Elimination of parasites by their host seems to be rare. VAN EMBDEN (1931) mentions that the parasite *Cephalonomia quadridentata* Duchaussoy can be damaged by its hosts, the larvae of *Ptinus tectus* Boield and of *Sitodrepa panicea* L. EVENHUIS (1958) communicates elimination of the parasite *Aphelinus mali* Hald. by its host, *Eriosoma lanigerum* Hausm. BISCHOFF (1927), quoting REUTER (1913), records aggressive behaviour of the larva of *Cicindela* against its parasite *Methoca.*

The ovipositing act of *Exenterus* failed in a number of cases (table 1). Apparently, these failures have been due to a rapid bending backwards of the larvae when being jumped upon. I could not establish whether the attacks of *Sturmia* on *pini* could be repelled by the responses of the larvae.

Recently it has been emphasized by VARLEY and EDWARDS (1957) that, for a proper understanding of a host-parasite interaction, a detailed knowledge of parasite behaviour is needed. It follows from the above observations that for that problem the behaviour of the host may be important as well.

b. The influence of the larval displays on the behaviour of the attacking parasites

A parasite after having detected a host, has to approach its victim to seek a position from which the final act—placing an egg—can be executed. I shall call this the pre-oviposition phase. In this phase, *Exenterus* and Tachinid X avoid releasing the displays of the sawfly larvae by moving slowly and carefully, *Sturmia* stays motionless after having alighted on a needle in the vicinity of a colony. During the next phase—the actual oviposition—Tachinid X keeps to its stealthy behaviour. In contrast, *Exenterus* and *Sturmia* obviate the responses of the larvae by attacking so rapidly that their reaction comes too late.

One might be inclined to suppose that these behaviour patterns have evolved in the parasites as an adaptation to the high responsiveness of the sawfly larvae. If this were true, one would not expect similar patterns to occur in parasites with hosts in which such a responsiveness is lacking.

Three categories of hosts can be distinguished: *a.* hosts—like eggs

and pupae —that neither respond to parasites moving in their vicinity nor to oviposition; *b.* hosts—like caterpillars—which do not respond as long as a parasite approaches but fight back, by rapidly bending backwards, as soon as it touches them; *c.* hosts—like the sawfly larvae studied—which respond both to approaching and to attacking parasites.

It is known that in the pre-oviposition phase parasites of eggs or pupae walk over their hosts; oviposition may last as long as 12 minutes here (DE FLUITER, 1934; SALT, 1935; HASE, 1925; JACOBI, 1939; THALENHORST, 1939). I could only find a few data on the second category. In the pre-oviposition phase, the Tachinid *Parasetigena segregata* Rdi. walks round its host, *Lymantria monacha* L. (PRELL, 1915). *Carcelia obesa* Zett., a Tachinid that parasitizes various species of caterpillars on pine, walks or flies round a host after having detected it (HERREBOUT, pers. comm.). Thus, these Tachinids behave in the pre-oviposition phase strongly different from the parasites of *Diprion*. However, they share with the latter the rapid ovipositing act.

These comparative data show that cautious behaviour in the pre-oviposition phase is typical for the parasites of the sawfly larvae, and thus may have evolved as a consequence of the capability of these larvae to respond to visual stimuli from moving enemies. In contrast, the rapid attack of the sawfly parasites need not necessarily be considered as a special adaptation to the responsiveness of their hosts as the same rapid way of attack is found in parasites of caterpillars which do not respond to visual stimuli. Perhaps in both cases the rapidity of the attack gives the host less chance to prevent oviposition by bending backwards.

c. *The influence of properties of the larvae and cocoons on the host stage chosen by the parasites.*

Another possible protection against offensive larvae would be to attack the host in its egg or cocoon stage. With this idea in mind I have compared the number of parasitic species (Hymenoptera) infecting the eggs, larvae and pupae (cocoons) of *Lymantria monacha* L. (Lepidoptera), *Panolis flammea* Schiff. (Lepidoptera) and of *Diprion* spec. These numbers are based on records of resp. FAHRINGER (1941), ESCHERICH (1931) and ESCHERICH (1942). Since Tachinids parasitize larvae only, they could not be included in the comparison.

Fig. 7 shows that the result is in agreement with what we could expect if our assumption were true: whereas in *Lymantria* and in *Panolis* most parasites are adapted to the larval stages, adaptations to cocoons reach a peak in *Diprion*. The number of egg parasites is small in all these cases.

To me it seems plausible that in general larvae will give better opportunities for parasitation than pupae. The larvae are often bigger and less hidden than the pupae and, as there is competition between different kinds of parasites, it will be of advantage to use the earlier stage as host, provided at least that there is no great difference in mortality between larvae and pupae. Consequently, it looks to me that there must have been special reasons for the *Diprion* parasites to avoid

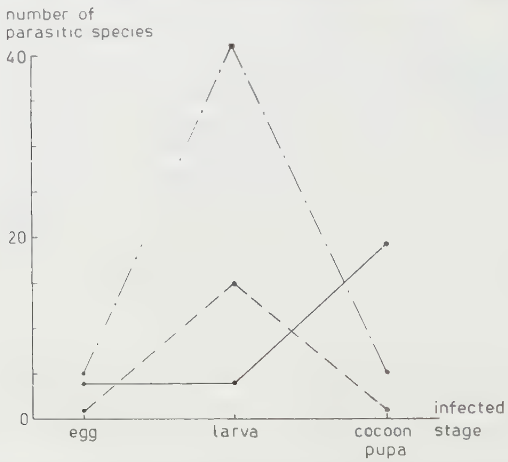


Fig. 7. Comparison of the number of parasitic species (Hymenoptera) infecting the eggs, larvae or pupae (cocoons) of *Lymantria monacha* L. (—·—), *Panolis flammea* Schiff. (– –) and *Diprion* spec. (—), after records of resp. Fahringer (1941), Escherich (1931) and Escherich (1942).

the larvae as hosts. This suggestion is supported by the fact that the few parasites using *Diprion* larvae are all specialists. This even applies to two species of Tachinidae, although, in general, parasites of this group are very polyphagous and only four species are known to be monophagous (Escherich, 1942). As suggested above, a reason why parasites avoid the sawfly larvae might be that the larvae are offensive. But, of course, also other agents may have contributed to the phenomenon under discussion. For instance, the sawfly larvae might be relatively unsuitable as food for the larvae of parasites, a possibility that could not be investigated within the scope of this paper.

III. CONSPICUOUSNESS OF THE LARVAE

Chapter II has shown that the gregarious larvae can bluff birds, at least temporarily, by stretching and jerking. In contrast, the birds were not intimidated by the U-bend of the solitary species. Therefore, at

first sight the gregarious larvae seem better protected. It might be, however, that the solitary species with their less elaborate displays are able to compensate a possible stronger predation by, for instance, a higher fertility. Another possibility is that each species has its own manner of preventing predation by birds; the protective factors involved might be quantitatively as well as qualitatively different from species to species. I have attempted to analyze and compare this latter possibility for the various species studied. My report on this investigation can be started best with an attempt to compare the species as to conspicuousness. I will deal separately with four components contributing to the total conspicuousness of the larvae: 1. the displays; 2. the spacing out; 3. the colour pattern and 4. the size of the larvae.

1. DISPLAYS

Variations among conspicuousness of the displays can be due: 1. to qualitative differences in form; 2. to quantitative differences in the tendency to respond to external stimuli.

To the human observer the conspicuousness of the form increases in the order U-bend, stretching, jerking. Although a U-bend makes a larva contrast with the needle (Plate I, 1; III, 1), to us this reaction appears much less striking than the other forms of display. This is probably also true for birds, for in the experiments given in chapter II the birds have never been scared by the U-bend.

To study the quantitative differences between the species I have undertaken a series of experiments.

First I investigated which characters of an approaching bird release each of the three responses mentioned in the six species studied. For the sake of standardization I used no real birds, but imitations of visual and mechanical stimuli from the bird. These artificial stimuli were applied to the different species of larvae in series of experiments under standard conditions. The experiments also give an answer to the question whether differences in responsiveness to these stimuli exist between the various species.

The larvae used in the experiments were in their last instar. They were placed on small pines or on cut pine twigs.

The visual stimulus consisted of swinging a pencil 2 cm in front of a larva, in a plane perpendicular to its axis, downwards, upwards and downwards again over a stretch of 3 cm at a velocity of 25 cm/sec. approx. The mechanical stimulus was a tap with the pencil on the twig, 15 cm away from the larva.

The experiments on the effect of visual stimulation had been

preceded by pilot experiments in which possible tactile effects resulting from the moving pencil were tested; as no differences were found between experiments in which the swinging was performed either behind a glass plate or without such a protection, the latter condition was used in the final experimental series to make the set-up as simple as possible. Care was taken that during mechanical stimulation no visual cues of the stimulating object could reach the larva.

The simplicity of this technique implies a certain amount of variation in the strength of the stimuli applied. However, I am convinced that these variations are only slight, and, moreover, they will be equally distributed over the different species.

Each single solitary larva was tested two or three times at two hour intervals. Gregarious larvae have always been tested within the colony. Of such a colony in each experiment an arbitrary member was chosen beforehand as the test animal. Colonies were never disturbed again before they were completely at rest; here the interval was at least 45 minutes.

The completeness of the U-bend obviously increases with the angle over which the frontal part of the body is raised. This is supported by the fact that a more intense bending can be brought about by repetition of the disturbing stimulus. Therefore, I have used the size of this angle as a measure of the intensity of the response. I shall call the response "full" when the angle is 60° or more and "incomplete" when it is smaller.

The results are laid down in tables II and III and in fig. 8. A rough survey of the quantitative data already suffices to answer the first question whether the different responses are released by different stimuli. In all species the U-bend is released by mechanical stimuli. Only in the solitary species visual stimuli can also release it. In the gregarious species, however, visual stimuli exclusively elicit stretching and jerking. In *pini* the visual stimulus applied released stretching far more often than jerking, whereas in *sertifer* jerking occurred in more than 90 % of the experiments, in 54 cases alone and in 35 cases after stretching. In a few cases *sertifer* responded with stretching only.

If a mechanical stimulus is applied to a colony when the larvae are already stretching or jerking, they immediately shift to a U-bend. On

PLATE III

Fig. 1. *Diprion nemoralis* Ensl. Responding larva. (Photograph by "Centrale Fotodienst", University Groningen.)

Fig. 2. *Diprion nemoralis* Ensl. Feeding larva. (Photograph by "Centrale Fotodienst", University Groningen.)

PLATE III

1

2

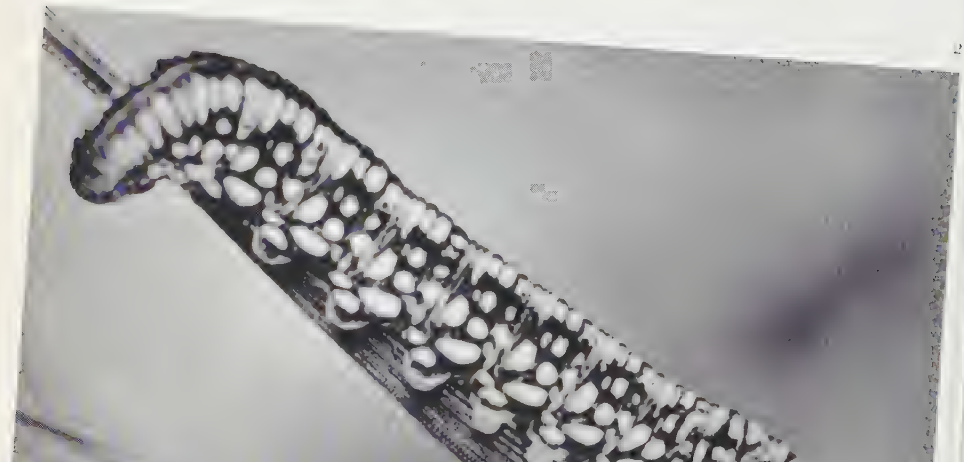


TABLE II

Responsiveness to visual and mechanical stimuli. In the second column: n. = number of experiments in which the result mentioned was obtained; perc. = percentage of total experiments

Species		Effect of Visual stimulus full U-bend	Visual stimulus incomplete U-bend	Visual stimulus no response[1]	Mechanical stimulus full U-bend	Mechanical stimulus incomplete U-bend	Mechanical stimulus no response[1]
virens	n.	0	1	69	0	2	76
	perc.	0 %	1.4 %	98.6 %	0 %	2.6 %	97.4 %
frutetorum	n.	4	37	114	36	34	61
	perc.	2.6 %	23.8 %	73.6 %	27.5 %	25.9 %	46.6 %
simile	n.	11	19	5	22	4	3
	perc.	31.4 %	54.3 %	14.3 %	75.9 %	13.7 %	10.4 %
nemoralis	n.	66	17	26	85	4	6
	perc.	60.6 %	15.6 %	23.8 %	89.5 %	4.2 %	6.3 %
pini	n.	47 responses[2]		17	25	12	5
	perc.	73.4 %		26.6 %	59.6 %	28.6 %	11.8 %
sertifer	n.	95 responses[2]		1	297	5	53
	perc.	99.0 %		1.0 %	82.8 %	1.4 %	14.7 %[3]

[1] "No response" in most cases means "stops feeding". [2] Specification in table III. [3] Moreover, *sertifer* responded to mechanical stimulation in 3 cases with jerking, in one case with stretching.

TABLE III

Responses in *pini* and *sertifer* to visual stimulation

Species		Effect of visual stimulation: stretching	stretching followed by jerking	jerking	no response
pini	n.	40	2	5	17
	perc.	62.5 %	3.1 %	7.8 %	26.6 %
sertifer	n.	6	35	54	1
	perc.	6.2 %	36.5 %	56.3 %	1.0 %

the contrary, when a visual stimulus is given to gregarious larvae that have already assumed a U-bend, this attitude is retained, the only effects are a temporal increase of the mouth drop and an oriented

PLATE IV

Fig. 1. *Diprion nemoralis* Ensl. Feeding larva. (Photograph by Mr. K. P. van Kempen.)

Fig. 2. *Diprion simile* Htg. Feeding larva. (Photograph by "Centrale Fotodienst", University Groningen.)

movement of the head towards the new stimulus. These observations suggest a dominating influence of the U-bend on the other patterns; it is remarkable that on the U-bend, which is normally not oriented towards the stimulus, under these circumstances an oriented component is superimposed.

A more detailed consideration is necessary for the second problem: the estimation of the responsiveness of the different species. I have approached this problem along two lines: 1. by comparing the frequency, and 2. by comparing the intensity of the reaction to visual and mechanical stimulation.

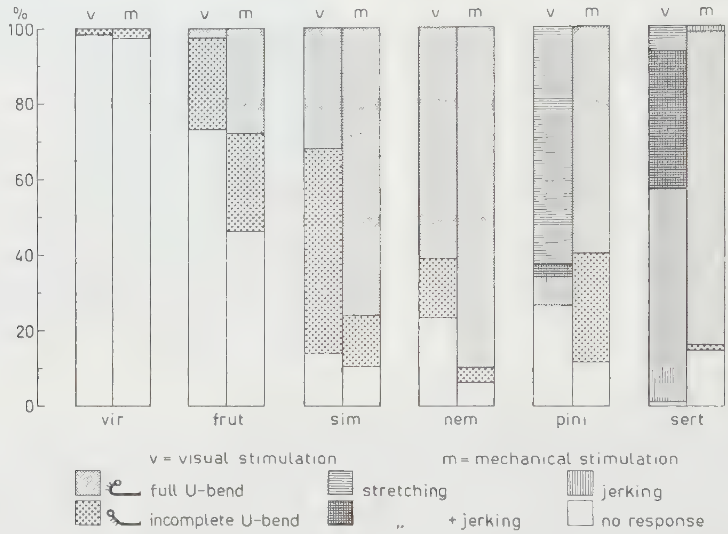


Fig. 8. Responsiveness to visual and mechanical stimuli. Percentages as in table II and III.

Ad 1. The diagrams in fig. 8 show specific differences between the percentage responses. The statistical analysis consisted in comparing in different combinations of two species the proportions between the number of times stimulation has been effective and ineffective, by calculating the adjusted X^2-values (see for the formula used SNEDECOR, 1953, p. 199). The results for visual and for mechanical stimulation are given in table IV. For both types of stimulation it is clear that *virens* has the lowest responsiveness. The responsiveness in *frutetorum* is significantly[1] higher than in *virens* but lower than in the other species. No significant differences were found between *simile*, *nemoralis* and *pini*.

[1] The criterion of significance has been taken at the $P < 0.05$ level.

With regard to a possible difference between this group and *sertifer* the results obtained are contradictory. With visual stimulation *sertifer* has responded significantly more than the members of the group, with mechanical stimulation, however, it responded less frequently than *nemoralis* and equally often as *pini*. These inconsistencies might be due to shortage of observations; the present material ony justifies the conclusion that responsiveness in *sertifer* is not different from that in *simile*, *nemoralis* and *pini*.

The results of these experiments agree with the observations in chapter II. The larvae of *virens* and *frutetorum* which were never seen to react to a stalking *Exenterus*, also showed a low responsiveness in the present experiments. Conversely, the many unsuccessful stalkings of *Exenterus* against *sertifer* agree with the high responsiveness of *sertifer* under experimental conditions.

Ad 2. The diagrams in fig. 8 also show differences between species in the intensities of the U-bend. As mentioned above, two stages have been distinguished: the incomplete and the full U-bend. The proportions between the numbers of times each of these was obtained I compared in different combinations of two species by calculating the X^2-values (table IV).

TABLE IV

X^2- and P-values for interspecific differences in responsiveness to visual and to mechanical stimulation. Significant differences are shown by P-values in heavy type

	Comparison of numbers of responses and non-responses after				Comparison of numbers of full and incomplete U-bends after			
	vis. stim.		mech. stim.		vis. stim.		mech. stim.	
Species compared	X^2	P	X^2	P	X^2	P	X^2	P
virens-frutetorum	18.3	**<0.01**	53.6	**<0.01**				
frutetorum-simile	40.4	**<0.01**	11.5	**<0.01**	5.99	**0.02**	7.39	**<0.01**
simile-nemoralis	0.9	0.35	1.04	0.31	16.7	**<0.01**	2.2	0.15
nemoralis-pini	0.05	0.82	0.5	0.50			15.9	**<0.01**
simile-pini	1.3	0.26					1.53	0.22
pini-sertifer	22.6	**<0.01**	0.09	0.77			59.3	**<0.01**
nemoralis-sertifer	21.2	**<0.01**	4.15	**0.04**			1.35	0.25
simile-sertifer			0.16	0.69				

When reacting to visual stimulation, *simile* showed a significantly greater number of full U-bends than *frutetorum*. The reactions in *nemoralis* were more often of a high intensity than those in *simile*. To a mechanical stimulus *simile* reacted usually with greater intensity than *frutetorum*. The difference in intensity between the responses of *nemoralis* and *simile* is not statistically significant. In the experiments *pini*

responded with a significantly lower number of full U-bends than *nemoralis*. The greatest number of high intensity reactions to mechanical stimulation was obtained from *sertifer;* the data for *sertifer* differ significantly from those of *pini*, but not from those of *nemoralis*.

Both the distribution of response frequencies and of response intensities show that the responsiveness in *frutetorum* is lower than in the group *simile*, *nemoralis*, *pini* and *sertifer*. The few results obtained from *virens* do not permit any conclusion on the distribution of its intensities.

In fig. 9 a schematic representation is given of the results of all calculations laid down in table IV. The species studied can be classified into three categories of responsiveness. On the basis of the data of fig. 8 I call the responsiveness in *virens* very low, in *frutetorum* rather low and in the other species high.

If we go into details, fig. 9 also shows that comparison of response frequencies does not always lead to exactly the same conclusion as comparison of response intensities. It lies outside the scope of this paper to discuss the causes of these minor inconsistencies. They might have disappeared if my material would have been greater. On the other hand, the assumption that all criteria by which one can measure what I have called "responsiveness" have to give an exactly similar picture is premature as long as we have not reached a full understanding of the causation of the complex of behaviour items underlying this "responsiveness".

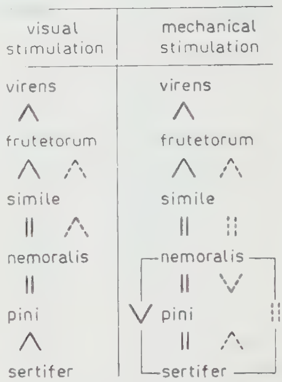


Fig. 9. A schematic representation of the interspecies comparisons made in table IV. Symbols in continuous lines refer to comparisons of response frequencies, those in broken lines to comparisons of response intensities.

In fig. 8 it is striking that with one exception the reaction to a mechanical stimulus was stronger than that to a visual stimulus, with regard to frequency as well as to intensity. To test the significance of these differences they were subjected to a X^2-test in table V. All differences in intensity proved to be significant ($P < 0.01$). Those in frequency are significant in *frutetorum*, *nemoralis* and *sertifer*. Although I do realize that I cannot compare the respective strengths of the visual and mechanical stimuli presented, these data seem to me to suggest a greater susceptibility of the larvae to the latter kind of stimulation. This would fit the already mentioned observation that in the gregarious species the, mechanically released, U-bend cannot be abolished by visual stimulation.

It is known that movement of a prey helps a bird to detect it (CARRICK, 1936; DE RUITER, 1952). Therefore, the more frequently and the more intensively a species of prey responds to an approaching bird, the more

TABLE V

X^2- and P-values for intraspecific differences in responsiveness to visual and mechanical stimulation. Significant differences are shown by P-values in heavy type

Species	Comparison of numbers of responses and non-responses		Relative frequency of responses is highest after	Comparison of numbers of full and incomplete U-bends		Relative frequency of the full U-bend is highest after
	X^2	P		X^2	P	
frutetorum	20.6	<**0.01**	mech. stim.	17.7	<**0.01**	mech. stim.
simile	0.01	0.90	equally effective	11.3	<**0.01**	mech. stim.
nemoralis	10.5	<**0.01**	mech. stim.	8.8	<**0.01**	mech. stim.
pini	2.48	0.12	equally effective			
sertifer	12.5	<**0.01**	vis. stim.			

often its individuals will be found by birds. Thus the differences in responsiveness established above mean at the same time differences in conspicuousness to birds. However, the species which have been called highly responsive differ partly in the form of their displays. I have already stated that the U-bend, which in the solitary larvae is the sole display, is less conspicuous than stretching and, particularly, than the rhythmical jerking of the gregarious larvae. This, apart from the effect of gregariousness discussed below, further increases the difference in contrast between the needles and, on the one hand, *pini* or *sertifer* larvae and, on the other hand, the larvae of *nemoralis* and *simile*.

The experiments show a relatively high degree of susceptibility of the larvae to visual stimuli. Structural and physiological properties of the larval eyes will be dealt with in a separate paper.

2. SPACING OUT

It cannot be doubted that the gregariousness of *pini* and *sertifer* strongly intensifies the conspicuousness of these larvae to birds. This applies especially to *pini* because its colonies are often compactly aggregated by day (Plate II, 2). Furthermore, the colonies of both *pini* and *sertifer* cause a strong defoliation, so that the larvae are freely exposed and thus the more conspicuous.

Looking for functions of gregariousness would be the more justified if the larvae would prove to possess special behaviour patterns favouring aggregation. For this reason I made the following study of the mechanisms that bring and keep the larvae together.

I have called *pini* and *sertifer* gregarious because their larvae form groups of from about 20 to as many as 100 members, all concentrated upon a relatively small number of neighbouring needles. These groups

arise because each female wasp deposits its eggs on some adjoining needles. Now it seems quite sure that each group of newly-emerged larvae would be doomed to dispersion if no mechanisms to keep the larvae together would exist. SZYMANSKI (1913) supposed that reactions to abiotic stimuli would play a main role in the group formation of larvae of *Hyponomeuta evonymella* and *Arye ustulata;* a positive attraction between the larvae he thought of no importance, a conclusion that has been questioned by DEEGENER (1922). GREEN (1954) is somewhat more explicit regarding the role of abiotic stimuli in the grouping of the larvae of *Neodiprion banksianae* and *N. lecontei.* These larvae are strongly photopositive. Therefore, GREEN supposes that a phototaxis leads the larvae automatically towards the end of their twig; in this way the top would become the common feeding place.

It may be that a positive phototaxis in gregarious larvae plays a certain role in keeping the larvae together. Yet I believe that its effect is unimportant, particularly in larvae living on pine. Plants in general and pine in particular often show a rather dense network of foliage in their periphery which implies that various sites in the tree or plant are equivalent as regards light conditions. This would easily cause dispersion of the larvae if light were the sole orienting factor. As, in general, colonies are compact, I am inclined to assume that in all gregarious larvae the group is maintained through a positive attraction between its members. I shall show that this is true at least for *pini* and *sertifer*.

When looking carefully at a *sertifer* colony, we find as a striking feature that often one needle out of a bundle of two is occupied by various larvae—3 or 4 at the top of the needle and some larvae resting on its lower parts—whereas the second needle bears one larva or is not occupied at all (fig. 10). Below I shall call a group of larvae on one needle a sub-group. The distances between neighbouring sub-groups ranges from 0.5 cm up to about 2 cm.

Fig. 10. Asymmetrical arrangements of *sertifer* larvae over the needles.

Such asymmetrical arrangements suggest that the larvae seek the company of other larvae. As, however, tufts can also be found where the arrangement over adjoining needles is more symmetrical, we have to face the possibility that the overall distribution is due to chance only. To check this I placed a *sertifer* colony on a twig of a low pine tree with long needles which permitted the formation of large sub-groups.

During 3 successive days I determined the distribution of the larvae over the needles by making sample counts every other hour in the daytime. The number of larvae in the sub-groups ranged from 1 to 10; fig. 11 gives the frequency distribution.

If the distribution over the needles had been at random, it has to fit a Poisson distribution. However, when sampling I did not know the

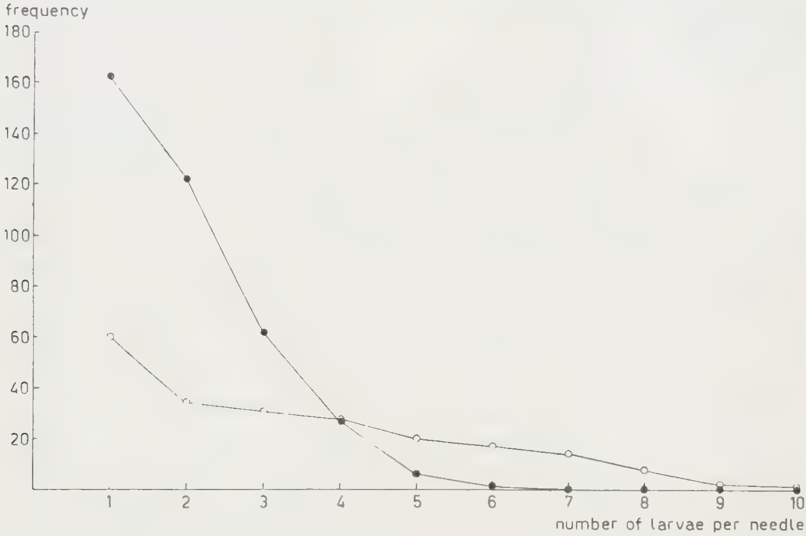


Fig. 11. Distribution of *sertifer* larvae over the needles.
○—○ Frequencies observed
●—● Poisson frequencies when m = 1.5 (see the text).

exact boundaries of the colony and, consequently, no more the number of needles unoccupied by chance. Thus the question whether the frequencies found correspond to a Poisson distribution can be approached indirectly only. The highest frequency is found in the class "1 larva per needle", the frequencies for the classes "2 per needle", etc. decrease gradually. This can hold for a Poisson series (see SNEDECOR, 1953, for the relative frequencies of the different classes) if the mean number of larvae per needle is smaller than 2.

However, for any value of this mean smaller than 2 the computed Poisson series deviates systematically (and significantly) from the series found: the frequencies found for the lowest classes are too low for a Poisson series, those of the highest classes are too high. As an example the computed Poisson series with m(ean) = 1.5 has been plotted in fig. 11. This systematic deviation of the series observed from the approximative Poisson series strongly suggests that the larvae are

inclined to form a greater number of relatively large sub-groups than might be expected in a chance-distribution.

The tendency to form relatively large sub-groups is also shown by the following evidence. Besides the arrangements of the larvae, I scored in the *sertifer* colony under discussion, how many larvae were feeding on each needle. Fig. 12 shows the relationship between feeding activity

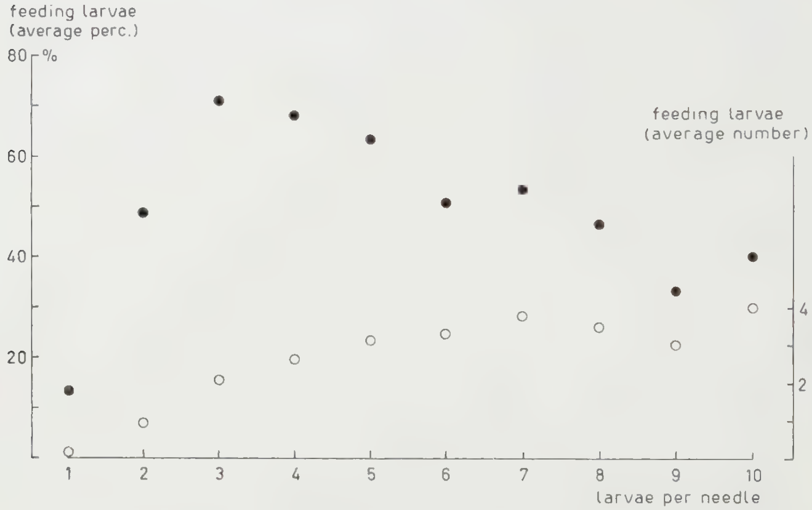


Fig. 12. Relationship in a *sertifer* colony between the frequency of feeding behaviour and the number of larvae per needle.
○ number of feeding larvae
● percentage feeding larvae.

and the number of larvae per needle, by expressing (a) how many and (b) what percentage of the larvae present were feeding.

We find that only part of the larvae were feeding at any moment. However, the average number gradually increased with the size of the sub-group. Two explanations are possible: 1. feeding is inhibited when a larva lacks the company of other larvae; 2. larvae when in need of food seek a feeding place amidst other larvae. The first possibility is supported by a finding of Long (1953) that the larvae of *Plusia gamma* L. exhibit a higher feeding activity when bred in crowded cultures than when kept solitary. That the *percentage* of feeding larvae gradually decreased in the sub-groups with 4 to 10 larvae can be easily explained. The top of a needle permits the simultaneous feeding of 3 or 4 larvae. Consequently, the percentage of feeding larvae can reach a maximum in the sub-groups with 3 and 4 larvae and has to decrease in the larger sub-groups. However, this would imply that in the large sub-groups a

number of "surplus" larvae would have started feeding if they had found an open site at the top. But then it is surprising that such larvae did not move to free feeding places but became completely inactive on the lower parts of the needle. This speaks for the explanation (2), suggesting a positive attraction between the larvae, that causes crowding on a single needle.

Curiously enough, the inactive larvae did not start feeding on the lower parts of their needle, though this would seem technically possible as young larvae often start gnawing at some distance from the needle top. Apparently the stimuli of the larvae which feed at the top are able to inhibit in the surplus larvae not only searching for a feeding place but also the feeding act itself.

Asymmetrical arrangements, like in fig. 10, are also recognizable in colonies of *pini*. They are less evident than in *sertifer*, however, owing to another behavioural pattern, typical for *pini*, and making the colonies still more compact. During night, when the larvae are loosely aggregated, they feed intensively. In the course of the morning most larvae stop feeding, leave their feeding places and take up a new position on the lower parts of the needles and on the twig. In this way a rather dense cluster of non-feeding larvae is formed (Plate II, 2). In the afternoon or towards the evening the larvae resume feeding at the needle tops. Fig. 13 demonstrates the diurnal feeding rhythm in a *pini* colony. Plotted are (a), as a measure of feeding: the rate of defaecation, measured as the number of faecal pellets produced per hour, in the period between 22 h. and 6 h. and in 2 hour periods between 6 h. and 22 h.; (b), the average temperature.

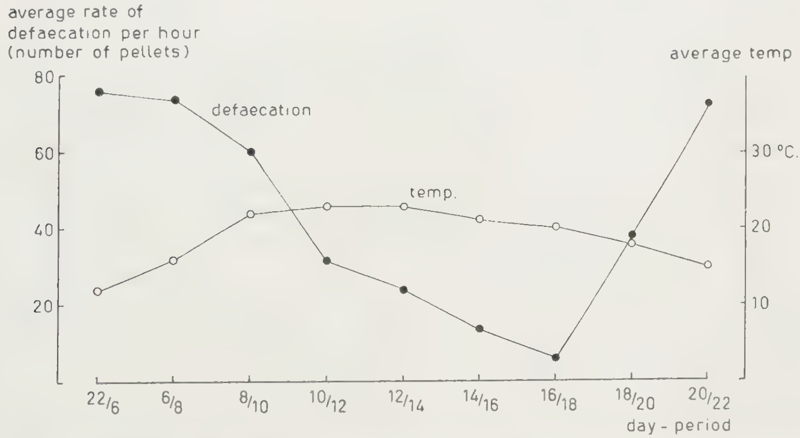


Fig. 13. 24 hour feeding-rhythm in a *pini* colony, measured by the average production of faecal pellets per hour in various day-periods.

I have seen in the field that interruption of feeding coincides with the formation of clusters. Apparently it is owing to its cluster-forming habit that *pini* does not feed by day and, consequently, has adopted the

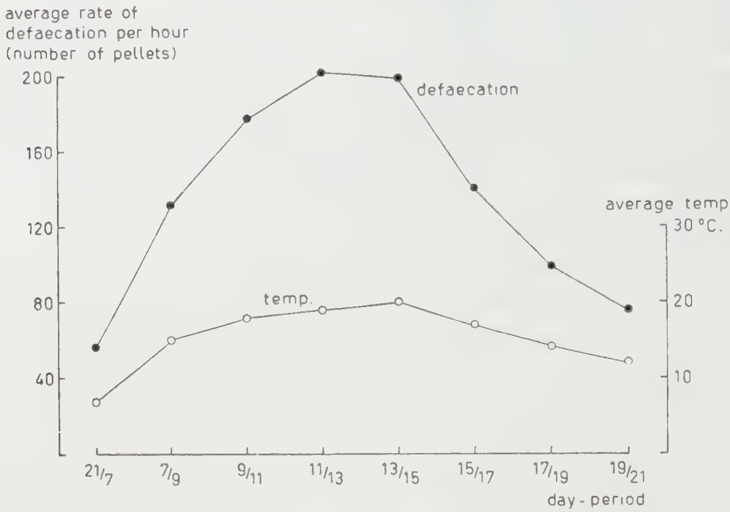


Fig. 14. 24 hour feeding rhythm in a *sertifer* colony. Compare fig. 13.

nocturnal feeding habit. This suggestion can be supported by a reference to *sertifer*. These larvae which do not retreat in a cluster show a feeding rhythm opposite to that of *pini:* they feed during the day and are inactive at night (fig. 14).

Above I have stated that the forming of sub-groups is based upon a positive attraction between the larvae. The use of mutual attraction is unmistakable when a colony of *pini* or *sertifer* is on the move to a new feeding place. Moreover, the stimulus then used for communication is easy to identify. This may be illustrated by a brief record which I made of the migration of a *sertifer* colony. This colony had nearly defoliated its twig when some larvae in its periphery started to walk. Having covered a distance of 2 cm, they arrived at a place where four twigs branched off. One larva followed a twig over 1 cm, then turned its head successively to the left and to the right and returned to the colony. A second larva chose the same twig. When it arrived at the place where the first one had turned, it also made sideways movements with its head and finally went back, like its predecessor. The next larva passed this critical point but turned 1.5 cm from the main-branch. Now this spot became the turning-point for other larvae. This turning at a certain point by some larvae, and shifting of the turning-point to a some-

what more distant spot by others occurred repeatedly. Fig. 15 shows schematically all turning-points on the four twigs. The distances estimated in centimeters between the main branch and the turning-points are given above or at the left of the twigs. The figures near the arrows indicate the number of larvae that turned.

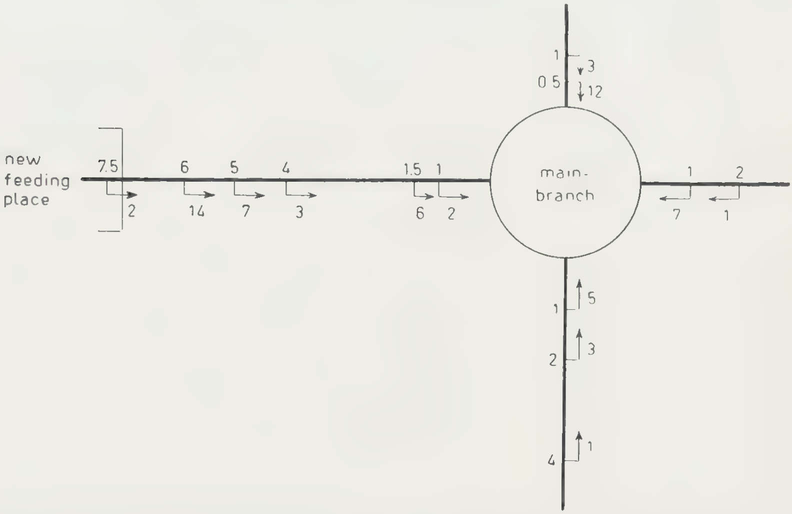


Fig. 15. Migration of a *sertifer* colony towards a new feeding place. Explanation in the text.

Most larvae turned at the same point as their immediate forerunner. This suggests that each larva makes a scent trail which can be perceived by followers; these turn when the trail stops. I consider, therefore, the sideways movements of the head which are performed before turning as a searching for stimuli from other larvae. Some pioneers go on for a while and turn at a new point. The result is that the marked route gradually lengthens while the contact between the larvae is constantly maintained.

In my example one gets the impression, that the left side-twig was accidentally more often visited by pioneers than the other twigs. The stronger scent here may have caused the larvae to follow this trail more and more, with the consequence that the trail on this twig became particularly long, and thus ultimately led to a new feeding place.

I have observed migrations of *sertifer* colonies over distances up to 2 meters.

In the foregoing description the accent fell on the performances of the colony as a unit since I could not follow the activity of the separate individuals. To obtain an idea of the share which a single individual

can have in the searching for a new feeding place, I have carried out some experiments of the following type. I transferred a larva from its colony to the middle of a 0.5 cm broad piece of wood. Then I recorded the crawling of the larva. Fig. 16 shows the result of such an experiment with a *pini* larva; experiments with *sertifer* gave a similar picture.

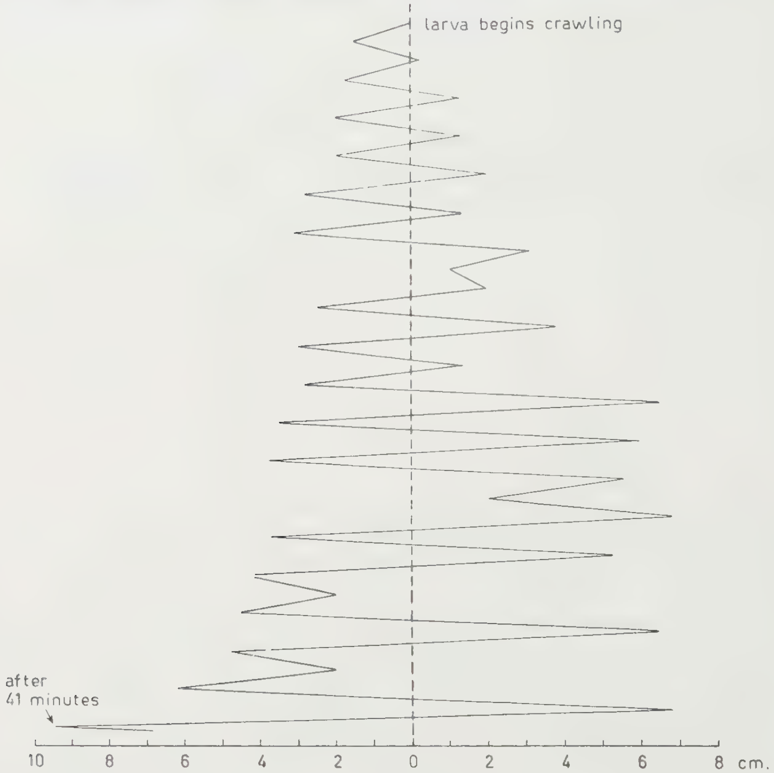


Fig. 16. Crawling of a *pini* larva on a 0.5 cm broad piece of wood. The larva starts with crawling to the left, turns and goes back along the same track, turns again etc., so gradually making its trail longer both to the left and to the right. In order to visualize this crawling to and fro, the tracks which actually overlap each other, have been parted by drawing them in a graph with a time scale on the ordinate.

The larva crawls along a narrow path to and fro and gradually lengthens its track to both sides (it must be noted that in other cases the lengthening was less symmetrical than in the given example). In some cases two successive turning-points coincide exactly, in other cases the larva turns before arriving at the end of its trail, more often it passes its latest turning-point a little. Thus we find the gradual lengthening of the scent trail also in the migration of one individual. The rate

of lengthening was low in these experiments, but, of course, it will be higher when more larvae take part.

It follows from these experiments that, when a larva is out of touch with its colony—for instance after moulting— it will have a good chance to rejoin the colony by crawling to and fro along its own scent trail. It would be of importance for searching if the larvae were negatively geotactic; although I have not tested this thoroughly, occasional observations make likely that this is true. Such a negative geotaxis is known in caterpillars (GÖTZ, 1936; DE RUITER, 1956). It makes the area which must be searched on horizontal twigs smaller, and consequently the larvae will meet more often.

In similar experiments *virens* larvae crawled completely at random without making systematic turns.

Above I have considered orientation to a scent trail as a basis for the crawling to and fro of isolated *pini* and *sertifer* larvae. It must be mentioned, however, that DE RUITER and VAN DER HORN (1957a and b) studying the crawling of caterpillars of *Smerinthus ocellata* L., placed on a horizontal table in a dark room with one fluorescent source of light, have found an alternation of short periods (1 minute to at least 10 minutes) in which a larva crawls towards the light with periods in which it moves in the opposite direction. I have not looked for similar short-term oscillations in the sign of the phototaxis in *Diprion* larvae. Yet for the following reasons I do not believe that, if present, they could provide a satisfactory explanation of the crawling to and fro in my experiments. First, I have carried out the experiments during daylight in a room with one window; the lath, used for crawling, was laid parallel to this window. Hence, changes in the direction of crawling cannot have been due to changes in the sign of phototaxis. Second, my experiments show a more or less regular increase of the track of the larva; in contrast, DE RUITER and VAN DER HORN did not find such an increase in the lapses of time between successive oscillations.

It may be asked when, after having been isolated, a gregarious larva starts searching. We know that a colony migrates to a new feeding place when its twig is defoliated. Then apparently, searching is induced by hunger. Now it might be that an isolated larva also needs lack of food as a stimulus for searching. This, however, is not the case. When we transfer a *Diprion* larva of one of the solitary species to another needle, it soon starts feeding there. The same happens when we transfer a *sertifer* or a *pini* colony to another feeding place. When, however, we isolate one *sertifer* or *pini* larva from its colony, transferring it somewhere to another needle, it begins crawling. The tendency, in such larvae, to move about may be exemplified by my experience that a cage, used for the isolation of a *pini* or *sertifer* larva and supplied with sufficient food, must be tightly closed in order to prevent escape of the isolated larva. It may be concluded, therefore, that such larvae start searching independently of the presence of food. Thus, whereas in the sub-groups of the colonies the surplus larvae, finding the top of the needle fully

occupied, do not crawl to another needle, we find that isolated larvae begin crawling independently of food conditions. This accentuates that the behaviour of the gregarious larvae serves the maintenance of their groups.

I did not study what factors induce searching after isolation. Certainly the absence of contact with other larvae will play an important role.

On the strength of the above observations it may be concluded that the colonies of *pini* and *sertifer* become integrated thanks to an appropriate behaviour pattern: 1. In moving to a new feeding place, the larvae keep contact by using scent trails as a means of communication. 2. The forming of relatively large sub-groups is due to a positive attraction between the larvae, complemented by an inhibition of feeding in larvae that have no place at the top of the needle. 3. Isolated larvae react, independently of food conditions, to the absence of contact with other larvae with searching behaviour, that enables them to catch up with the colony.

Surely, this picture is incomplete. For instance, it might be that, despite a positive attraction between the larvae, a large colony splits up into two smaller groups. However, this rarely occurs. For an explanation a detailed knowledge about the contact between the various sub-groups seems necessary.

Gregariousness is a factor enhancing conspicuousness, but this is not its sole function. It also leads to a greater watchfulness (chapter II). Moreover, it seems certain that the greater a colony the greater its intimidating effect will be and the more successful its active defence against small attackers like parasites (chapter II).

Furthermore, Fintelmann, in a paper of 1839, has pointed to a physiological effect of crowding. He claimed that the temperature in a cluster of *pini* larvae surpassed that of the surrounding air: a cluster temperature of 10.5° R was measured when the air showed 7° R. His observation is of interest since a higher temperature might increase the responsiveness of the larvae to approaching enemies. Mosebach-Pukowski (1938), who has found a similar temperature effect in the colonies formed by the larvae of *Vanessa io* and *V. urticae*, states on the strength of experiments that the higher temperature speeds up larval growth. The same may hold for pini, but seems impossible in sertifer because these larvae touch each other not at all or only slightly (see fig. 10).

The fact that *pini* and *sertifer* have evolved an elaborate behaviour pattern to promote aggregation indirectly suggests that gregariousness is protective, one way or another. Apparently, the advantages of crowding are so great that they can outweigh such drawbacks as the fact that in *sertifer* and in *pini* group formation interferes with feeding and thus retards growth, or the fact that crowding facilitates the spread of virus infections via infected food (Smith and Williams, 1958).

3. COLOUR PATTERN

The human observer can easily distinguish differences in the contrast individuals of the different species studied make against a background of needles.

Virens has a real disruptive pattern, its ground colour is green while two longitudinal white bands give a somatolytic effect when the larva is sitting in its normal position on the needle (Plate v, 2). The preponderantly green *frutetorum* is also difficult to detect for the human eye (Plate v, 1). *Nemoralis* and *simile* are much easier found, because their spotted pattern of light and dark elements, without being disruptive, stands out against the green needles (Plate III, 1 and 2; IV, 1 and 2). In *sertifer* the greyish green body colour does not contrast with the needles, only the black head is more striking (Plate I, 1). Finally, the larvae of *pini* that show individual varations from yellowish green to almost orange are usually very conspicuous (Plate I, 2; II, 1 and 2).

But is it allowed to generalize these human impressions to birds? There are arguments to assume so. Species of prey have been presented to birds by several investigators in series of quantitative experiments, partly in situations where they contrasted against the background, partly in situations where they were less conspicuous. Thus KETTLEWELL (1955) found that when the Peppered Moth, *Biston betularia* L., was presented to tits on different backgrounds, the birds took the moths in an order of conspicuousness similar to that gauged by the human eye. Similar experiments have been carried out by ERGENE (cited by PORTMANN, 1956), working with larvae of *Oedipoda* and of *Acrida*, and by SUMNER (a very detailed survey of his experiments can be found in COTT, 1957) who used the fish *Gambusia patruelis* as a prey.

In all these experiments, prey that to the human eye seemed to be better camouflaged were caught in significantly smaller numbers. DE RUITER's results (1956) also show that birds have difficulties in finding a prey which to humans looks inconspicuous. He found that, when countershaded caterpillars were placed in an inverted resting attitude on leaves—and thereby were made more conspicuous to the human eye—birds also detected them more easily. The opinion of physiologists that there is very little difference in colour vision between birds and man (PUMPHREY, 1948) again speaks for similarity in the visual information perceived. This view is also expressed in a paper by MOSTLER (1935). Therefore, it seems justified to conclude at least that, in accordance with the human impressions, the colour pattern of *pini*, *nemoralis* and *simile* must be more conspicuous to the avian eye than that of *virens* and *frutetorum*.

It must be remarked, however, that although there is evidence that birds are strongly handicapped in finding non-contrasting prey, this implies by no means that they are unable to detect a camouflaged prey. Here a result of DE RUITER (1952) must be stressed. He found the camouflage of stick caterpillars to give protection only as long as the test birds had not found and consumed the caterpillars. Once this had

happened by accident, the birds learned to peck at sticklike objects (finding only sticks probably reversed this learning). Some birds (Jays) were even able to distinguish the stick caterpillars from sticks in spite of a high resemblance. Comparably, TINBERGEN (1959) has found that the highly cryptic larva of *Lymantria monacha* is caught in considerable numbers by titmice.

Such observations show that also in highly camouflaged prey protection cannot be supposed to be absolute. Consequently it is of great importance to a prey to improve its concealing properties as much as possible in the course of evolution. Moreover, even an animal which is camouflaged to some extent will profit from obtaining other means of protection.

Results to be discussed in chapter IV will prove that birds can distinguish different species of prey. The observations of DE RUITER in particular suggest that for such a discrimination a small difference in colour pattern suffices. For corroboration I may also refer to MÜHLMANN (1934) who found that red painted mealworms could be discriminated by birds from red ones with a white spot. It may further be mentioned that, according to MOSTLER (l.c.), birds do not discriminate the harmless *Syrphidae* from the unpleasant *Vespa* specimens. This is no wonder since these forms are strikingly similar in their appearance. It seems probable that the *Syrphidae* have mimicked the *Vespa*-form under the agency of avian predation. In view of DE RUITER's observations it is conceivable that a close similarity is necessary to prevent visual discrimination by birds.

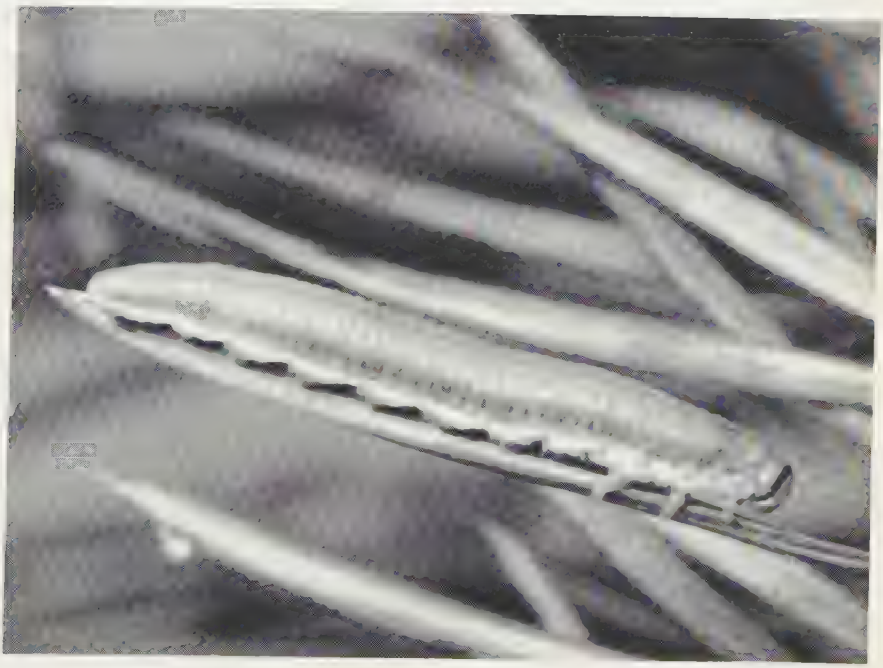
I have made some experiments to test the discriminating capacity of birds by giving a Great Tit the choice between two similar looking species of prey. In a series of 15 experiments I presented each time one larva of *Panolis flammea* Schiff. and one, of equal size or larger, of *virens*. Both kinds of larvae resemble each other as long as *Panolis* is not in its last feeding stage (Plate VI, 2). The *virens* specimens used measured 15 to 22 mm, those of *Panolis* 10 to 21 mm. The clear difference in the shape of the legs I tried to reduce by laying the larvae on a side with the legs in the shadow (I experimented in a room with window light from one side). A remaining difference was the colour of the head: brown in *Panolis* and green with a little black in *virens*. To obtain results more quickly the already existing preference for *Panolis*

PLATE V

Fig. 1. *Diprion frutetorum* F. (Photograph by "Centrale Fotodienst", University Groningen.)

Fig. 2. *Diprion virens* Kl. (Photograph by "Centrale Fotodienst", University Groningen.)

PLATE V



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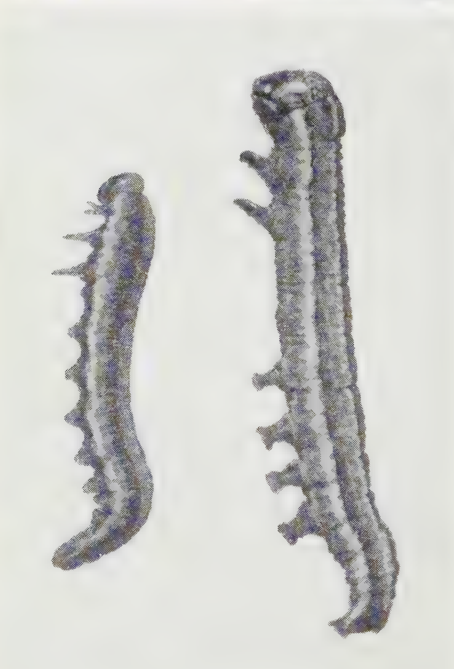

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was increased by smearing the hidden side of the *virens* larvae with mouth-drop liquid of *pini*. Although it is very unlikely that the birds could have used smell I excluded this possibility by smearing mouth-liquid on the feeding tray near the two larvae.

Table VI shows the result obtained after 5 preliminary experiments. Obviously the Tit could discriminate in spite of the relatively small visual differences between the alternatives.

TABLE VI

Species	Average length	First taken by the Tit
Panolis	14.7 mm	14 ×
virens	19.0 mm	1 ×

The above information is surely insufficient for a definite conclusion. Yet the arguments presented strongly support the opinion that insectivorous birds may make use of details in the coloration of a prey to recognize it specifically. This idea is not new. It has been suggested already by SWYNNERTON (1915) who spoke in this context of a "distinctive' coloration. Later in this paper I shall implicate this specific recognition of a prey in a consideration of selective feeding by birds.

COTT (l.c.) rates the discriminating capacities in birds considerably lower: he is of opinion that even crude resemblances suffice to delude birds. COTT bases this statement on some observations of MOTTRAM (1918). These observations apply to mistakes made by birds when attacking prey-like inanimate objects. I believe that their importance has been overestimated by COTT. First, I want to raise the objection that these observations were incidental; they do not prove that in general a bird makes errors in similar conditions. Moreover, the kind of observations may be criticized. MOTTRAM and COTT refer for instance to the observation that a Spotted Flycatcher mistakes floating inanimate things for insects. I doubt, however, whether such an example may be taken as a measure for the perception of optical details by birds. It is sure that in a Flycatcher movement has a relatively high value among the external factors releasing attack; in contrast, the importance of optical details seems to be rather subordinate. But it has been found in ethological studies that a sharp difference must be made between what an animal can perceive and what it actually uses in a given case (N. TINBERGEN, 1951; BAERENDS, 1956). So, though only one detail from the total sensory information may suffice to release a certain activity, the release of

PLATE VI

Fig. 1. *Panolis flammea* Schiff. Larva in resting attitude. (Photograph by Mr. K. P. VAN KEMPEN.)

Fig. 2. At the left: full-grown larva of *Diprion virens* Kl. At the right: larva of *Panolis flammea* Schiff. in the last but one feeding stage. (Photograph by "Centrale Fotodienst", University Groningen.)

another response in the same animal may require a much greater number of sensory details (see BAERENDS, 1950). Consequently, observations like those brought forwards by COTT are in no way conclusive to the capacities of birds to distinguish visual details. As an illustration I may refer to the behaviour of Herring Gulls in two different situations. Herring Gulls learn to distinguish their own young and can recognize them amidst many other; their ability in this respect is astonishing to the human observer. There is some evidence that the dark blotches on the head of the chickens play a role in this recognition (N. TINBERGEN, 1953). In contrast, the gulls do not know their own eggs. They even accept models that are very different from normal eggs (BAERENDS, 1954).

It is likely that at least birds which usually hunt non-moving prey, like larvae, must use their full visual discriminating power in hunting. The records given above show that these capacities are remarkably good.

4. SIZE

The size of a prey surely affects its conspicuousness for birds. The larvae of *frutetorum* and *sertifer* are relatively small: I found resp. 111 and 141 mg as their maximal weights. The larvae of *pini* and especially of *nemoralis* are considerably bigger, with maximal weights of resp. 231 and 285 mg. Those of *simile* and *virens* are intermediate as to size, with maximal weights of 189 mg and 186 mg respectively. However, the relatively large size of *virens*—and of *virens* only—is counterbalanced by a disruptive colour pattern. Thus it may be stated that the contribution of size to conspicuousness is highest in *nemoralis* and *pini*, somewhat lower in *simile* and again lower in *virens*, *sertifer* and *frutetorum*.

5. CHARACTERIZATION OF OVERALL DEGREE OF CONSPICUOUSNESS

The properties of the species with respect to the criteria for conspicuousness have been summarized in table VII. The contrast of the coloration with the needle colour has been qualified with the terms contrasting or non-contrasting. The degree of responsiveness has been called high, rather low or very low. I call stretching and jerking highly conspicuous, the full U-bend conspicuous and the incomplete U-bend inconspicuous. A useful qualification of the contribution of size to conspicuousness could not be found; therefore the maximal weights are mentioned only. A record of the effect of aggregation seemed unnecessary.

The last column of the table indicates our final characterization of the larvae. Though this way of assessing conspicuousness would be far from satisfactory from a quantitative point of view, it is at least sure that three groups of clearly different degrees of conspicuousness can be distinguished.

TABLE VII

The relative conspicuousness of the larvae

Species	Criteria of conspicuousness						Final characterization
	maximal weight of the larvae in mg	gregarious or solitary	colour pattern of the larvae: rough description	colour pattern of the larvae: contrast with the needle colour	degree of responsiveness	the form of the displays	
pini	231	colonies are often compactly aggregated	preponderantly yellow to yellowish green	contrasting	high	especially stretching and jerking are highly conspicuous	highly conspicuous
sertifer	141	gregarious	head black, for the rest greyish green	non-contrasting	high	as in *pini*	highly conspicuous, particularly when jerking
nemoralis	285	solitary	spotted pattern of light and dark grey and of yellow and white	contrasting	high	U-bend is conspicuous by being most often of full intensity	conspicuous
simile	189	solitary	spotted pattern of yellow and dark blue	contrasting	high	as in *nemoralis*	conspicuous
frutetorum	111	solitary	preponderantly green	non-contrasting	rather low	U-bend is fairly often incomplete and therefore rather inconspicuous	inconspicuous
virens	186	solitary	a disruptive pattern of green with two longitudinal white bands	non-contrasting	very low	U-bends are rare, incomplete and inconspicuous	inconspicuous

Pini and *sertifer* have been called highly conspicuous since the eye-catching effect of their responsiveness and their displays is supported by the effects of aggregation and in *pini* by its coloration and size.

Nemoralis and *simile* are called conspicuous. Although these solitary species are surely considerably less eye-catching than the colonial larvae, the striking effect of their easily elicited full U-bends, supported by the effect of a contrasting, non-disruptive colour pattern and of a relatively large size, makes them contrasting with the needles.

Virens has been characterized as inconspicuous since its very low responsiveness scarcely interferes with the concealing effect of its colour pattern. *Frutetorum* is more responsive. Despite this, its degree of conspicuousness approaches far more that of *virens* than that of *nemoralis* and *simile*, due to its cryptic colour, its small size and, moreover, to the frequency and form of its U-bend. Therefore it has also been called inconspicuous.

It must be stressed once more that the above qualifications are based upon human impressions. But it follows from what has been said in the separate sections that it seems fully justified to assume that the differences distinguished apply to birds as well.

IV. ATTRACTIVENESS OF THE LARVAE

To obtain a relative measure of the appreciation birds have for each of the six species of sawfly larvae, I presented to wild and to captive birds various assortments of anaesthetized specimens, often together with other insect larvae, on a homogeneous coloured feeding table.

A preliminary series of experiments ("Feeding Experiments I") was aimed at a comparison of the frequency at which the different species are taken. A second series ("Feeding Experiments II"), besides from giving more detailed information, moreover provides data on the sequence in which different, simultaneously presented larvae have been carried away, thus making a more direct measure of the preference of the birds possible.

1. FEEDING EXPERIMENTS I

A. Material and technique

The experiments have been carried out with (a) wild birds: six breeding pairs of the Great Tit (*Parus major* L.) in a pine wood and one breeding pair of the Blue Tit (*Parus caeruleus* L.) in a town garden; (b) caged birds: one Chaffinch (*Fringilla coelebs* L.), caught as an adult, and two Great Tits, caught just before they would have left the nest.

For the experiments with wild birds I placed in a number of territories of Great Tits a feeding tray, painted evenly with a light greenish grey (surface area 20 × 20 cm, height 1 m). The birds were attracted to the trays by baiting these with mealworms. Experiments on a tray were started only when the tray was regularly frequented by one or both members of a breeding pair. A hide made observation from a distance of 2 m possible. In order to prevent ants—a serious nuisance—from visiting the tray, I smeared a non-drying glue on the supporting leg over a distance of 20 cm.

The experiments with captive birds were carried out in cages (measuring 158 × 78 × 78 cm, each containing one bird), on the bottom of which the feeding-tray was placed. The staple food (seeds and a ground mixture of vegetables and animal food) was not removed during the experiments.

The larvae used in the experiments I had to collect in the wood by shaking trees, a very laborious and time consuming work. In order to keep enough time for the experiments, I frequently had to be satisfied with a poorer collection of prey specimens (particularly with regard to the less common species) than would actually have been required for an ideal experimental design.

In the series of experiments I offered either solitary *Diprion* larvae (*virens*, *frutetorum*, *nemoralis*, *simile*, 5 8 simultaneously), or gregarious larvae (*pini*, *sertifer*), or non-Tenthredinid larvae (which will be specified when the results are presented). The larvae were in their last instar, except for the caterpillars of *Bupalus piniarius* L.

Usually the birds paid several consecutive visits, taking one prey or more each time. I recorded for each species (independently of the number and kind of other larvae simultaneously present) the number of times a specimen was exposed to the birds and the number of times this was taken. Often all larvae were carried away within 5 to 10 minutes. When, after an experiment had lasted for half an hour, one or more larvae were still left on the tray, I added one or two mealworms (a highly appreciated food, as I knew from preliminary trials) to make sure that the birds had noticed the experimental set-up and that the result had not to be attributed to an insufficient tendency in the birds to collect food In all cases these mealworms have been taken within 5 minutes, usually very soon after presentation.

The larvae were anaesthetized with ether in order to prevent them from crawling away and to exclude differences in behaviour. This procedure was made as short as possible since the colour of the larvae, particularly green, was easily modified by the narcotic.

B. Results and conclusions

The results have been summarized in table VIII which gives the number of larvae presented and consumed in the three groups distinguished: non-sawfly larvae, solitary sawfly larvae and gregarious sawfly larvae. The following conclusions can be drawn:

1. The wild birds took the non-Tenthredinid larvae more frequently than the solitary sawfly larvae (adjusted $X^2 = 18.5$ and $P < 0.01$, when comparing the numbers consumed and refused of these groups), and

TABLE VIII

The relative attractiveness of the sawfly larvae and of non-Tenthredinid larvae to wild and to caged birds

Species of prey		Wild birds: Great Tits	Wild birds: Blue Tits	Wild birds: Total	Caged birds: Great Tits	Caged birds: Finch	Caged birds: Total
non-	numbers offered	90[1])	14[4])	104	104[6])	24[9])	128
Tenthredinid	numbers consumed	85	14	99	98	24	112
larvae	perc. consumed			95.2			87.5
solitary	numbers offered	39[2])		39	98[7])	13[10])	111
sawfly	numbers consumed	26		26	93	13	106
larvae	perc. consumed			66.7			95.5
gregarious	numbers offered	88[3])	29[5])	117	94[8])	2[11])	96
sawfly	numbers consumed	36	2	38	36.5	2	38.5
larvae	perc. consumed			32.5			40.1

[1] *Larentia firmata* Hb., *Panolis flammea* Schiff., mealworm. [2] *virens.* [3] *sertifer, pini.* [4] mealworm. [5] *sertifer.* [6] *Larentia firmata* Hb., *Cacoecia piceana* L., *Panolis flammea* Schiff., *Bupalus piniarius* L. [7] *virens, frutetorum, nemoralis, simile.* [8] *sertifer, pini.* [9] *Larentia firmata* Hb., *Panolis flammea* Schiff. [10] *virens, simile.* [11] *pini.*

the latter more often than the gregarious larvae of *pini* and *sertifer* (adjusted $X^2 = 12.8$, $P < 0.01$). These differences are also expressed by the percentages consumed in the three categories: non-Tenthredinid larvae 95%, solitary sawfly larvae 66% and gregarious larvae 32%.

2. The caged birds took as many of the non-Tenthredinid as of the solitary sawfly larvae (87% and 95% respectively; adjusted $X^2 = 3.78$, $P = 0.06$). Like the wild birds they consumed only a low percentage (40%) of the gregarious larvae; the differences between the numbers consumed and refused of, on the one hand, the gregarious larvae, and on the other hand the solitary sawfly and the non-Tenthredinid larvae, are significant (adjusted X^2 resp. 72.9 and 53.6, in both cases $P < 0.01$).

3. There is no significant difference between the frequency at which the wild and the captive birds took the gregarious larvae (adjusted

$X^2 = 1.10$, $P = 0.30$) and the non-Tenthredinid larvae (adjusted $X^2 = 3.24$, $P = 0.08$), but relatively more solitary *Diprion* larvae have been consumed by the caged than by the wild birds (adjusted $X^2 = 20.1$, $P < 0,01$).

C. Discussion

In general, I found the wild birds quite reluctant to come to the trays. In June and July of 1951 six feeding trays were visited by tits but only after their young had hatched; my earlier trials had no success. In the summer of 1953 and 1954 the birds did not visit the tables at all. In one case it was found that a bird only visited a tray to obtain a badly needed kind of food: a Crested Tit would take bits of egg shell (source of scarce but indispensable calcium), but never touched animal food on the tray, not even big *Panolis* caterpillars. These observations suggest that caterpillars and other larvae were only taken from the trays by hungry birds. This may explain that, whereas in the wood the gregarious larvae run a very low risk of predation (according to TINBERGEN, about 1000 times lower than *Panolis* caterpillars), in the experiments the wild birds have consumed no less than 32% of these, evidently unattractive, larvae.

2. FEEDING EXPERIMENTS II

The experiments have been particularly designed to detect more detailed differences in appreciation of the four solitary *Diprion* species. The birds had to make a choice between a number of larvae of different kinds, presented simultaneously.

A. Material and technique

I had planned to do these experiments with wild tits, but after a great many attempts had not given me any suitable opportunity, I decided to be satisfied with experiments on captive birds. I chose Chaffinches because they can stand caging very well and because their diet in summer, at least in pine wood, is comparable to that of tits (GLASS, pers. comm.). Two of the Chaffinches had been caught in the winter, four months before they were used in the experiments. The third was caught in a pine wood, five days before the start of the experiments.

To make sure that the Finches were acquainted with the various kinds of prey to be used, these species were regularly offered to the birds during four days preceding the experiments.

I presented larvae of *pini*, *sertifer*, *nemoralis*, *simile*, *virens*, *frutetorum* and, in addition, of *Panolis flammea* Schiff. (syn. *P. griseovariegata* Goeze) as a

non-Tenthredinid species, in the set-up already described for the "Feeding Experiments 1".

I would have prefered to compare the preference for the different species by presenting the birds each time two species only, in series of tests of all possible combinations. For this I would have needed a great number of larvae. However, the solitary *Diprion* larvae and particularly *simile* were so scarce that only small numbers of them could be collected. Hence I was compelled to design a technique which could provide conclusive results in a relatively small number of experiments. This led to an experimental set-up in which in each experiment two specimens of several species were presented simultaneously. Often six species were given when one of the seven was not available. Thus, when an experiment started, a bird had to choose among 12 or 14 larvae. These were arranged in 2 rows. The order of the different species in these rows I made different in each experiment to exclude the influence of a possible position-preference in the birds. The intervals in and between the rows were 1.5 cm. The rows covered an area of about 6 $\times$ 17 cm that could easily be overlooked by the birds. In a visit a bird never took more than one larva. Taking away of a larva meant thus that in the next visit the number of specimens between which the bird had to choose, had decreased with one.

The results will show that, in general, the birds have not taken the larvae at random. Already from the behaviour of the birds when approaching the tray one could see that they were carefully making a choice. Often they passed several larvae at short distance before taking the preferred one. Their selectivity is also shown by the fact that, after having obtained some experience, I could rather successfully predict in what sequence the larvae offered in an experiment would be taken.

Because of our limited supply of prey specimens, individuals of different size had to be used in the same test. This introduced an important complication for if the birds would have a preference for relative or absolute size of a prey, this would certainly interfere with a preference for particular species. Therefore, it is necessary to find out whether a size-preference exists in the feeding behaviour of the birds and whether such a preference is based on a judgement of the absolute size of two alternatives or of their size-ratio.

In 1949 I have carried out some pilot experiments with wild Great Tits, offering them mealworms of four different size-classes which I shall call a, b, c and d. The weight of the first three size-classes was respectively 0.33, 0.50 and 0.67 times that of d. The variation in weight within each class was very small. The birds had to choose between either two mealworms of class a and one of class d, or two of b and one of d, or one of c and one of d, a design I have chosen to limit the number of experiments

necessary to obtain statistically significant results. The alternative taken first was scored.

Table IX shows a discrimination for size only when the size-ratio was 0.33 or 0.5, not when it was 0.67.

More information about size-preference is provided by the feeding experiments described in this section, with larvae of sawfly and of *Panolis* as prey. I mentioned above that in these experiments I always presented two individuals of each species simultaneously. A number of these intraspecific pairs of alternatives showed the same size-ratios as used in the experiments with mealworms. There are two differences,

TABLE IX

Experiments on size-preference

Species offered	Weight-ratio of alternatives	Test birds	Alternative taken first (freq. observed) bigger	smaller	(freq. expected[1]) bigger	smaller	X^2 [2])	P	Bigger alternative preferred
ealworm	1:3	3 wild tits	34	6	13.3	26.7	48.2	<0.01	+
iprion + Panolis	1:3	3 caged finches	16	1	8.5	8.5	15.0	<0.01	+
ealworm	1:2	3 wild tits	21	9	10	20	18.1	<0.01	+
iprion + Panolis	1:2	3 caged finches	12	3	7.5	7.5	5.40	0.02	+
ealworm	2:3	2 wild tits	11	12	11.5	11.5	0.04	0.85	0
iprion + Panolis	2:3	3 caged finches	15	11	13	13	0.61	0.45	0

req. expected = the frequencies which might have been expected if the bird had no size-preference. In the experiments one ger and two smaller, or one bigger and one smaller prey were presented. [2] X^2 = sum of the ratios $\frac{\text{deviation square}}{\text{expected number}}$.

however. First, the larvae of *Diprion* and *Panolis* were, as a rule, bigger than the mealworms. Second, whereas the absolute difference in size between two alternatives of a particular size-ratio was constant in the mealworms, it was highly variable in *Diprion* and *Panolis*.

Nevertheless, it follows from table IX that the agreement between the results of the series with mealworms and those with *Panolis* and *Diprion* is satisfactory. This means that size-ratio was used as a criterion by the choosing birds. This conclusion is supported by the (specified) data from table X, showing that the results within a particular size-ratio were independent of the absolute size of the alternatives. Consequently, in dealing with the results I shall discuss differences in size between alternatives in terms of size-ratios.

Within the range used, absolute size must be at least less important as a criterion. From what is generally known of food preference in animals, one would expect the curve describing the appreciation for different sizes of prey to show an optimum which shifts in accordance with the bird's need for food.

In working out the results of the experiments, I have assumed that a bird, when taking a larva, always chose the most attractive prey. Consequently, I have—irrespective of how many larvae were present on the tray—always treated the data as if it were series of choices between two alternatives. So when, for instance, larva p was taken out of a group consisting of p, q, r, s and t, I have recorded that the bird made a choice between p and q, p and r, p and s and between p and t.

I have distinguished between intra- and interspecific pairs. Since the larvae I collected of each species were of very different sizes, both the intra- and interspecific pairs showed considerable differences in size-ratio. In the interspecific series, part of the specimens of species A were bigger than those of B, partly the reverse was true. Care was taken to choose the assortments offered to the birds in such a way that for the total of all presentations the variation of the size-ratios was the same in the intra- and interspecific pairs. Consequently, if the birds would have selected for differences in size only, the results with both types of pairs must be equal. If discrepancies occur, these must be ascribed to selection on other properties, which I shall take together here under the term "quality" of a prey. Quality, therefore, is the resultant of a bird's appreciation of taste, coloration (see JONES, 1932; BRUNS, 1954; HESS and GOGEL, 1954) and possibly, other larval characters as three-dimensionality (DE RUITER, 1956), degree of hairiness and shape, but size excepted. The final appreciation of a prey, the resultant of quality and size, I will call its attractiveness. The term palatability seems less appropriate here in view of its exclusive association with taste sensations.

To study the interference of the influence of quality with that of size, I have compared the results of the interspecific series with those of the intraspecific one (by means of the adjusted X^2-test) after combination of the data into six size-classes: with respectively weight-ratios of < 0.44 (size-class 1″ in table XI), 0.44–0.66 (size-class 1′), 0.67-1 (size-class 2a), 1.01–1.50 (size-class 2b), 1.51–2.25 (size-class 3′) and > 2.25 (size-class 3″). Class 2a and 2b lie below the value that was shown to play a part in the experiments on size preference (table IX). When insufficient data were available, all ratios smaller than 0.67 have been lumped together in a size-class 1, those greater than 1.5 in a size-class 3.

In principle I have tested all species of sawfly larvae against each other. However, only a small number of the rather rare *simile* larvae could be collected; to have sufficient data for statistical treatment, I compared this species with *virens*+*frutetorum* (which proved to be of about equal value). The data available on *pini* and on *sertifer* would permit a separate comparison with the other species. However, their

TABLE X

Result of the intraspecific series

Weight-ratio of the alternatives		Alternative chosen: bigger		Alternative chosen: smaller	
1.01–1.50		41		34	
1.51–2.25	> 1.5	40	72	13	20
> 2.25		32		7	

Specification:

Weight-ratio	Weight range of the bigger alternative: 0–100 mg			100–200 mg			200–300 mg			300–400 mg			400–500 mg		
1.01–1.50	**21**	19	(a)	**13**	13	(b)	**7**	2	(c)						
1.51–2.25	**15**	4	(d)	**16**	6	(e)	**8**	2	(f)	**1**	1	(k)			
> 2.25	**6**	2	(h)	**13**	3	(i)	**8**	0	(j)	**5**	1	(g)	**0**	1	(l)

Species	Specification of: a	b	c	d	e	f	g	h	i	j	k	l
frutetorum	**12**	**3**		**6**	**2**			**3**				
	9			1								
virens	**5**	**4**		**8**	**1**				**5**			
	6	4		2					1			
nemoralis		**3**	**3**		**9**	**4**			**2**	**3**		
		4	1		5	1		1				
simile		**2**							**1**			
		2			1				1			
sertifer	**2**			**1**	**1**			**1**	**1**			
	2	2										
pini	**2**		**2**					**2**				
Panolis		**1**	**2**		**3**	**4**	**1**		**4**	**5**	**5**	
	2	1	1	1		1	1	1	1		1	1
	21	**13**	**7**	**15**	**16**	**8**	**1**	**6**	**13**	**8**	**5**	**0**
	19	13	2	4	6	2	1	2	3	0	1	1

heavy type = bigger alternative chosen   normal type = smaller alternative chosen

value is so evidently inferior that, for the sake of brevity, I combined the data on these species and tested them with *virens+frutetorum* and with *nemoralis+simile*. In addition, *Panolis* has been compared with *virens* and with *frutetorum*.

B. Results and conclusions

The agreement between the results with the three Finches—one newly- and two long-caged individuals –was satisfactory enough to allow pooling of the data obtained with these birds.

Table x gives for the intraspecific series the data in the size-classes distinguished. In table xi I have compared these results with the corresponding data in various interspecific series. The name of a species in the last column of the table indicates a significant preference for this species; "equal" means that no significant difference was found.

The results have been summarized in fig. 17 which is particularly intended as a graphic demonstration of the regular trend in the data obtained. In this graph the curves 2 to 9 give the results for the interspecific pairs of alternatives, curve 1 represents those of the intraspecific pairs. In each size-class the number of times species A was preferred over B has been plotted as a percentage of the total number of choices the birds made in that class between A and B. In the size-classes 1 and 2a of the curves 2 to 9 the larvae A have been smaller than the larvae B (in only one single case of class 2a they were of equal size), in the other classes bigger. Similarly, in the classes 1 and 2a of curve 1 the percentages refer to the smallest alternative, for the rest to the biggest one.

When a curve lies below curve 1, this expresses—apart from statistical significance—that A is of lower quality than B. A higher quality in A causes a curve to run above curve 1. When the difference in quality between two species is relatively small, the curve will run at a short distance from curve 1. The greater the difference in quality the more will the curve diverge from curve 1 until it finally runs horizontally.

If the birds had taken the larvae only at random, any regularity would have been lacking in the curves of fig. 17. On the contrary, we find curve 2, 3, 5, 6 and, partly, 7 running more or less parallel to curve 1 which represents the intraspecific choices. This means that in the interspecific series concerned the gradual increase of the frequency of choices in favour of species A from size-class 1 to size-class 3″ must have been due to size preference. In addition, the distances between curve 1 and the curves 2–9 clearly demonstrate differences in quality; the horizontal course of curve 8 and 9 even shows a dominant influence

TABLE XI

Interspecific comparisons of quality

Species compared (A ←→ B)	Size-class	Ratio $\frac{\text{weight A}}{\text{weight B}}$	Result obtained		Corresponding result from table x (selection on size only)		X^2	P	Conclusion as to quality
			A chosen	B chosen					
virens (A)	1	< 0.67	3	8	20	72	0.001	0.98	equal
×	2a	0.67–1	23	18	34	41	0.84	0.38	equal
frutetorum (B)	2b	1.01–1.50	31	10	41	34	4.08	0.045	*virens*
	3′	1.51–2.25	23	0	40	13	5.18	0.02	*virens*
	3″	> 2.25	12	1	32	7	0.20	0.67	equal
nemoralis (A)	1	< 0.67	1	2					
×	2a	0.67–1	1	13	34	41	5.71	0.02	*frutetorum*
frutetorum (B)	2b	1.01–1.50	8	29	41	34	9.70	< 0.01	*frutetorum*
	3′	1.51–2.25	10	23	40	13	15.2	< 0.01	*frutetorum*
	3″	> 2.25	28	13	32	7	1.35	0.25	*frutetorum*
nemoralis (A)	1	< 0.67	0	10	20	72	1.50	0.23	equal
×	2a	0.67–1	2	15	34	41	5.22	0.02	*virens*
virens (B)	2b	1.01–1.50	4	37	41	34	20.7	< 0.01	*virens*
	3′	1.51–2.25	9	20	40	13	13.6	< 0.01	*virens*
	3″	> 2.25	14	13	32	7	5.53	0.02	*virens*
simile (A)	1	< 0.67	0	2					
×	2a	0.67–1	0	6	34	41	3.04	0.09	equal
virens + *frutetorum* (B)	2b	1.01–1.50	2	16	41	34	9.39	< 0.01	*vir* + *frut*
	3′	1.51–2.25	3	10	40	13	10.4	< 0.01	*vir* + *frut*
	3″	> 2.25	1	8	32	7	13.9	< 0.01	*vir* + *frut*

TABLE XI *(continued)*

Species compared (A ⟷ B)	Size-class	Ratio weight A / weight B	Result obtained A chosen	Result obtained B chosen	Corresponding result from table x (selection on size only)		X^2	P	Conclusion as to quality
pini + *sertifer* (A)	1″	< 0.44	0	17	7	32	2.04	0.16	equal
×	1	0.44–0.66	0	55	13	40	13.0	< 0.01	*vir* + *frut*
virens + *frutetorum* (B)	2a	0.67–1	0	97	34	41	52.4	< 0.01	*vir* + *frut*
	2b	1.01–1.50	0	110	41	34	74.0	< 0.01	*vir* + *frut*
	3′	1.51–2.25	0	69	40	13	74.2	< 0.01	*vir* + *frut*
	3″	> 2.25	0	52	32	7	62.0	< 0.01	*vir* + *frut*
pini + *sertifer* (A)	1	< 0.67	0	100	20	72	22.0	< 0.01	*nem* + *sim*
×	2a	0.67–1	1	48	34	41	25.4	< 0.01	*nem* + *sim*
nemoralis + *simile* (A)	2b	1.01–1.50	2	40	41	34	26.8	< 0.01	*nem* + *sim*
	3	> 1.50	1	12	72	20	23.6	< 0.01	*nem* + *sim*
Panolis (A)	1	< 0.67	6	8	20	72	1.89	0.18	equal
×	2a	0.67–1	10	8	34	41	0.27	0.62	equal
frutetorum (B)	2b	1.01–1.50	14	7	41	34	0.53	0.48	equal
	3′	1.51–2.25	15	1	40	13	1.53	0.22	equal
	3″	> 2.25	45	2	32	7	2.94	0.09	equal
Panolis (A)	1	< 0.67	9	13	20	72	2.51	0.12	equal
×	2a	0.67–1	5	10	34	41	0.33	0.65	equal
virens (B)	2b	1.01–1.50	7	16	41	34	3.21	0.08	equal
	3′	1.51–2.25	11	4	40	33	0.03	0.87	equal
	3″	> 2.25	23	16	32	7	3.94	0.049	*virens*[1]

[1] This result is somewhat flattered in favour of *virens*. In some experiments the test bird was evidently interested in *Panolis*, as soon as the experiment had started. Yet it took *virens* first, because in those experiments *Panolis* was so large that for a while it induced escape behaviour in the bird.

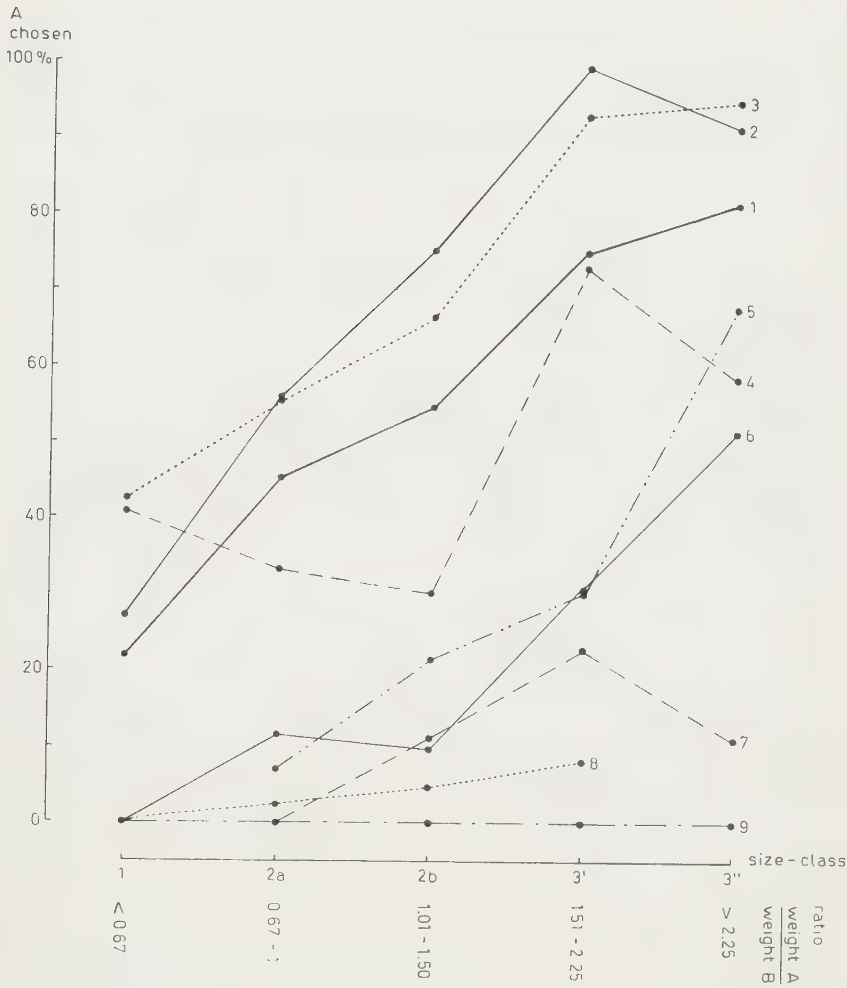

Fig. 17. A graphic summary of the results from table x and xi. Explanation in the text.

1 = conspecific alternatives
2 to 9 = A ⟷ B
2 = *virens* ⟷ *frutetorum*
3 = *Panolis* ⟷ *frutetorum*
4 = *Panolis* ⟷ *virens*
5 = *nemoralis* ⟷ *frutetorum*
6 = *nemoralis* ⟷ *virens*
7 = *simile* ⟷ *virens* + *frutetorum*
8 = *pini* + *sertifer* ⟷ *nemoralis* + *simile*
9 = *pini* + *sertifer* ⟷ *virens* + *frutetorum*

of a selection on quality over one on bigger size. We may say, therefore, that the test birds have chosen the larvae according to very definite norms. Consequently we consider the reliability of our methods proved.

The following conclusions may be drawn from the comparisons made:

A. With regard to quality only:

1. The quality of the gregarious larvae *pini* and *sertifer* is lowest of all: these larvae were always taken after *virens* and *frutetorum*, and apart from a few exceptions, also after *nemoralis* and *simile* (table XI and curve 9 and 8).

The birds have consumed so few specimens of *pini* and *sertifer* that a comparison between these species is not possible.

2. Curve 2, representing the comparison of *virens* with *frutetorum*, lies above curve 1, suggesting a higher quality of *virens*. However, this difference is significant in the size-classes 2b and 3′ only (table XI); no more shows the sum of the five X^2-values an overall preference for *virens* ($X^2 = 10.30$, d.f. $= 5$, $P = 0.07$). Therefore, I shall consider *frutetorum* and *virens* as equal.

3. The quality of *nemoralis* is lower than that of *frutetorum* and *virens* (table XI and curve 5, 6): both *virens* and *frutetorum* are significantly preferred in four size-classes.

4. The quality of *simile* is lower than that of *virens*+*frutetorum* (table XI and curve 7): the preference for the latter two species is significant in three classes.

5. Due to lack of data, a direct comparison of *nemoralis* and *simile* is not possible. However, in fig. 17 the curves 5, 6 and 7 almost coincide (the low value in size-class 3″ of curve 7 seems unreliable). This suggests that *nemoralis* and *simile* are of the same quality.

6. Although curve 3 runs above curve 1 and thus suggests *Panolis* to be of a higher quality than *frutetorum*, neither any of the classes in table XI nor the total of the X^2-values ($X^2 = 7.16$, d.f. $= 5$, $P = 0.21$) shows a significant difference.

7. Comparing *virens* with *Panolis* (table XI and curve 4), a higher quality of *virens* is found in 3″ only, but the result in this class is dubious (see the footnote of table XI). Therefore, it must be concluded that *virens* and *Panolis* are of equal value. This agrees with the earlier conclusions that the qualities in *virens* and *frutetorum* and those in *frutetorum* and *Panolis* are the same.

8. Summarizing, with regard to the appreciation by the birds the larvae of the six sawfly species can be arranged into three groups: *virens*+*frutetorum*, *nemoralis*+*simile* and *pini*+*sertifer*, with a relatively high, an intermediate and a low quality respectively.

This order of appreciation is also expressed in the following data: 24% of the *virens* and *frutetorum* larvae, consumed by the birds, was taken as number 1 or 2 in the experiments, 27% as number 3 or 4; of the *nemoralis* and *simile* larvae that were consumed 10% was taken as number 1 or 2, 16% as number 3 or 4; none of the gregarious larvae was consumed as number 1, 2, 3 or 4.

B. With regard to the interference of quality and size:

9. In size-class 3′ of the comparisons of *frutetorum* and of *virens* with *Panolis*, 15 out of 16 and 11 out of 15 choices have been in favour of *Panolis*, although the alternatives, according to conclusion 6 and 7, were of the same quality. This result shows that *Panolis* caterpillars become evidently more attractive than these alternatives as soon as they are about twice (1.51 to 2.25) as big as the latter.

Comparing *nemoralis* with *frutetorum*, size-class 3″ shows that 28 out of 41 choices were in favour of *nemoralis*. This means that the lower quality of *nemoralis* as compared with *frutetorum* can be fully compensated for if the larvae of the former species are more than 2.25 times bigger than *frutetorum* alternatives. It follows from conclusion 5 that the same probably applies to *simile* with regard to *frutetorum*.

A similar conclusion can be drawn when comparing *nemoralis* with *virens* (table XI and curve 6).

The horizontal course of curve 9 proves that the difference in quality between, on the one hand, *pini* and *sertifer* and, on the other hand, *virens* and *frutetorum* is so great that larvae of the latter two species are even preferred when the gregarious larvae are more than 2.25 times as big.

C. With regard to attractiveness:

10. The maximal weight I have found for *Panolis* is 440 mg, for *virens* 186 mg, for *frutetorum* 111 mg, for *nemoralis* 285 mg, for *simile* 189 mg, for *pini* 231 mg and for *sertifer* 141 mg. In combination with the conclusions sub B, this means that:

a. Panolis caterpillars are more attractive than full-grown larvae of *frutetorum* when their weight is higher than about 1.5×111 mg $= 167$ mg and more attractive than those of *virens* when their weight is higher than about 1.5×186 mg $= 279$ mg.

b. Full-grown larvae of *virens* are more attractive than those of *nemoralis* and *simile*. Full-grown *frutetorum* larvae too are more attractive than those of *simile;* however, they are less attractive than *nemoralis* larvae weighing from 250 mg (2.25 times the maximal weight of *frutetorum*) to 285 mg (the maximal weight of *nemoralis*).

c. The qualitively inferior *pini* and *sertifer* are too small for reaching the level of attractiveness of the other species.

The inferiority of the gregarious larvae is proved once more by the fact that of the specimens offered (with an average weight of 95 mg) only 29 (= 20 %) have been taken in the experiments. In contrast, of *virens*+*frutetorum* (average weight = 74 mg) 122 larvae (= 86 %) have been consumed, of *nemoralis*+*simile* (average weight = 141 mg) 72 larvae (= 85 %).

3. THE CAUSE FOR UNATTRACTIVENESS

From the foregoing sections it is clear that at least some of the sawfly larvae are not favourite species of prey. I have tried to find out why.

I knew from TINBERGEN that tits begin a more intensive predation upon *Diprion* species as soon as these larvae have spun their cocoon. This remarkable finding made me taste the larvae, in feeding and in cocoon stages, myself. I found that the feeding larvae of all species studied had a disagreeable resinous taste which, however, had almost disappeared in the cocoon stage. The nasty taste was found to be due to a liquid in two lateral diverticula of the fore gut. In the following experiments I have tested whether this liquid is also disagreeable to birds.

I do not know the chemical nature of the liquid. Since it tastes and smells like resin and is also very sticky, resin seems to be an important component. An account of the production of this liquid will be published later, in combination with an anatomical study of the lateral diverticula.

A. Material and technique

The test birds were three Chaffinches, caged separately.

In each experiment groups of three anaesthetized mealworms were offered to one bird, 20 times in succession, with intervals of at least an hour. These 20 tests were followed by 10 trials with mealworms, each smeared with 2 mouth drops of a sawfly larva. The drops were obtained by pressing with a pair of forceps the dorsal side of the thoracic segments 1 and 2 (where the diverticula are located) of a larva; each donor was used once.

By scoring latency of the attack on each of the mealworms I could test whether or not the liquid had decreased the attractiveness of the mealworms. As a control, I offered, after the last experiment with the smeared mealworms, three series of three mealworms each.

Drops of *pini* were used in experiments with two of the Finches, drops of *virens* in a series with the third Finch; in this latter series, however,

the number of experiments with normal mealworms was reduced to 15, those with smeared ones to 8. The donors were of equal size.

B. Results and conclusions

Figs. 18 and 19 show the average time elapsed between presentation and the attack on the first prey, between this attack and that on the second, and between the attack on the second and on the third prey. The latency was short with normal mealworms. Smearing did not delay

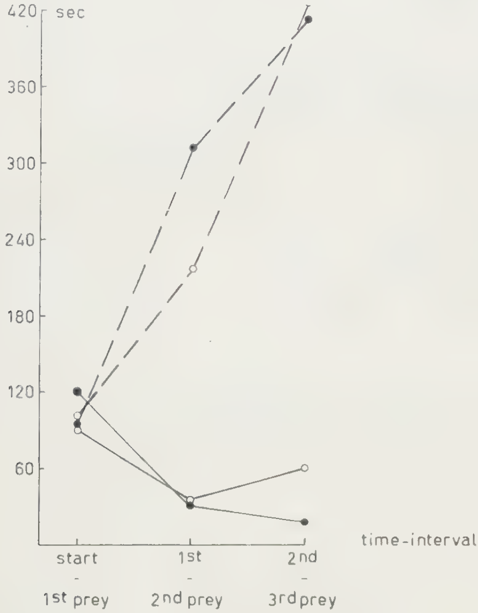

Fig. 18

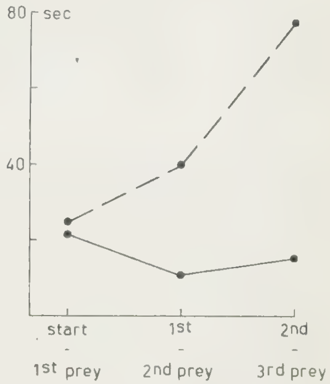

Fig. 19

Fig. 18. Smearing mouth-liquid of *pini* on mealworms delays the attacks of the birds
——— normal mealworms • Finch 1
– – – – smeared mealworms o Finch 2

Fig. 19. As fig. 18, but with *virens* as donor and test bird Finch 3.

the first attack. However, the divergence of the curves demonstrates that, in all three Finches, smearing caused a strong (and, according to table XII, statistically significant) delay of the attacks on the second and particularly on the third prey.

The normal mealworms which have been offered after the end of the experiments with smeared ones were again taken rapidly one after another. It may be concluded, therefore, that the liquid from the gut-

diverticula of both *pini* and *virens* had a repellent effect on the birds. This effect was not so strong, however, that the smeared mealworms were totally refused by the Finches. Only in one experiment, with Finch 3, one smeared prey was taken, whereas the other two were refused.

To human norms, the liquid in *frutetorum*, *nemoralis*, *simile* and *sertifer* is equally nasty tasting and sticky as that in *pini* and *virens*. It seems likely, therefore, that the attractiveness of larvae of all these

TABLE XII

A statistical comparison (t-test) of corresponding latencies in the experiments with normal and with smeared mealworms

Finch nr.	Comparison of time-intervals between the attacks on					
	1st and 2nd prey			2nd and 3rd prey		
	t	d.f.	P	t	d.f.	P
1	4.1	28	<0.01	5.1	28	<0.01
2	4.5	28	<0.01	4.3	28	<0.01
3	3.8	20	<0.01	4.7	20	<0.01

species is reduced by this liquid. The results in this section suggest that the liquid of *pini* has a stronger repellent effect than that of *virens* (compare figs. 18 and 19). Such a conclusion is not permitted, however, since I could press, as a rule, more liquid from *pini* than from equally-sized *virens* individuals. It must also be mentioned that responding larvae of *sertifer* produce more liquid on their mouth parts than those of bigger species as *virens*, *simile* or *nemoralis*. These facts draw attention to the possibility that the differences in appreciation, found in "Feeding Experiments II", are due to specific differences in the amount of diverticulum-liquid, independent of the larval size.

I have not studied whether also other properties support the unattractiveness of the larvae.

From many observations I know that an attacking bird usually directs its first peck at the head of its victim (see also BLEST, 1957). A bird will do this the more readily when potential victims like these sawfly larvae raise their head ostentatiously as a response to its approach. Now, it is important that a drop of liquid from the gut-diverticula appears exactly on the spot attacked (Plate 1, 2). Thus a larva achieves with its response that the protective value of the liquid is exploited as fully as possible.

V. THE NATURE OF THE PROTECTION OF THE SAWFLY LARVAE AGAINST BIRDS

In this chapter I shall try to combine the information detailed in the preceding pages into a coherent picture of the protection of the sawfly larvae against birds.

In chapters III and IV I have compared the species studied as to conspicuousness and attractiveness. The result is that on both criteria the same three groups of two species each can be distinguished: 1. *pini* and *sertifer*—most conspicuous and least attractive of all; 2. *nemoralis* and *simile*—intermediate on both scores; 3. *virens* and *frutetorum*—inconspicuous and most appreciated.

It is obvious that inconspicuousness may protect a larva from birds by reducing the chance of its being detected. On the other hand, animals with properties unpleasant to their enemies are protected only if they have some distinctive characters by which the predators can recognize them, without actually tasting them (COTT, 1957). In general, of course, the more a colour pattern contrasts with its natural environment, the more easily is it recognized. Hence, the fact that the combinations of unpalatability and conspicuousness and of palatability and camouflage are of general occurrence, is a strong supporting argument for these hypotheses.

The problem is to find out what are the respective shares of camouflage and unattractiveness in the protection enjoyed by each of the species studied.

According to TINBERGEN's field data, the average frequency of *pini* and *sertifer* in the food of tits is about 1000 times lower than that of *Panolis*, when the population densities of the sawfly larvae and the caterpillar are the same. This difference in risk must be due to inequality of combined effects of intimidation, detectability and attractiveness in the species compared.

I have concluded in chapter II that the displays of *pini* and *sertifer* can intimidate birds, but that this effect wanes as the birds gain experience with the prey. Yet TINBERGEN has never obtained any evidence, as far as I know, that experienced predators prey upon the gregarious larvae more intensively. Even during an outbreak, when the great number of encounters must have favoured the waning of the intimidatory effect, the low level of predation persisted (BESEMER, 1942)

Therefore, the low risk of *pini* and *sertifer* must be due to inconspicuousness and (or) unattractiveness. Now in chapter II I have argued that the gregarious larvae are highly conspicuous. In contrast, the *Panolis* caterpillar, the standard species in TINBERGEN's computations, avoids attracting attention. It is disruptively coloured with

longitudinal bands of needle-green and white. During day it does not feed, and hides its brownish head against the bark of the twig, thus acquiring the appearance of a needle bundle (Plate VI, 1). Moreover, the caterpillar does not respond to visual and tactile stimuli. Therefore, the possibility can be discarded that the low risk of *pini* and *sertifer* is due to concealment. The feeding experiments have proved the relatively low attractiveness of *pini* and *sertifer:* the wild birds have eaten 32 % of their larvae, the caged birds 40 % (exp. I) and 20 % (exp. II). One factor contributing to this unattractiveness is the content of the gut-diverticula.

It can be concluded that the gregarious larvae are protected against birds by their unattractiveness.

As to *nemoralis* and *simile*, I have mentioned that their coloration and their high responsiveness to disturbances make them relatively conspicuous among the needles (chapter III). Therefore, birds must detect them more easily than *Panolis* caterpillars. Despite this, TINBERGEN has found that in nature the risk of being attacked by a tit is about 33 times higher for a *Panolis* caterpillar than for a specimen of *nemoralis* or *simile*. We must conclude that tits consume only a small part of the *nemoralis* and *simile* larvae they actually find. Since the display of these two species has no intimidating effect (chapter II), only unattractiveness remains to be considered as a possible explanation of their low risk. It has indeed been shown in chapter IV that these larvae are relatively unattractive to birds, but less so than *pini* and *sertifer*. In nature the same must be true, since the risk of *pini* and *sertifer* is about 30 times lower than that of *nemoralis* and *simile*. This difference in unattractiveness may be due to a difference in amount of liquid in the diverticula of the gut (chapter IV, exp. III).

It is true that there is a gap in the evidence for the unattractiveness of *nemoralis* and *simile*, because these species have not been offered to wild birds. This is due to the fact that either wild birds were lacking when larvae were available, or the reverse. However, in exp. I (chapter IV) it was found that the wild birds consumed 32 % of the gregarious larvae which, undoubtedly, are highly unattractive in the wood. The birds took a higher percentage of the *virens* specimens offered (66 %), but this value is still considerably lower than the percentage reached by the non-Tenthredinid species (95 % consumed). So the wild birds demonstrated a certain degree of unattractiveness in *virens* as well. Since exp. II in chapter IV proved that *nemoralis* and *simile* were less appreciated than *virens* by the captive birds, it may be assumed that in the wood *nemoralis* and *simile* again are more unattractive than *virens*. This is the more probable because in exp. I the captive birds took more solitary sawfly larvae than the wild ones.

I conclude, therefore, that the fairly low level of predation upon *nemoralis* and *simile* is the result of a somewhat intermediate (but still considerable) level of unattractiveness.

For *virens* and *frutetorum* TINBERGEN has also found a low risk value in the field, about 45 times lower than that of *Panolis*. Intimidating displays are absent in *virens* and *frutetorum*. On the other hand, there is some evidence from experiments with wild birds for unattractiveness in *virens*, 66 % of which were consumed against 95 % of the non-Tenthredinid species and 32 % of the very unattractive gregarious larvae (exp. I). A further indication was the repellent effect of the liquid from the diverticula of the gut (exp. III). As regards *frutetorum*, tests with caged birds have shown that in quality it is equal to *virens*, but that its relatively small full-grown larvae (111 mg) are somewhat inferior to the bigger *virens* specimens (166-186 mg). Perhaps in the trees, where the birds see one larva at a time, size differences are a less important factor than in choice experiments. It may be, therefore, that the attractiveness of full-grown *frutetorum* and *virens* larvae scarcely differ. The finding of TINBERGEN that during his observations *virens* and *frutetorum* have never been selectively hunted by tits (in the terminology of TINBERGEN: the tits never have used a "searching image" of *virens* and *frutetorum*), also supports that both species are not favoured by the birds.

The conclusion that *Panolis* is more attractive than *virens* and *frutetorum* is in agreement with the data from chapter IV. It is true that there is no difference in quality among the three species, but *Panolis* is far bigger than the sawfly larvae (440 mg as opposed to 186 and 111 mg, resp.). In this context it must be stressed that, when *Panolis* is present in the wood, the tits have to feed many young: as a rule there are at least five in a nest, and even ten is rather common (LACK, 1958). The weight of the prey will be very important to these birds.

Another property that may protect *virens* and *frutetorum* is their inconspicuousness. One might even be inclined to suppose that the low predation upon these species must be due to this property alone, their cryptic appearance being too little distinctive to prevent erroneous attacks. However, I have argued in chapter III that a single visual detail of a prey already permits specific recognition. It therefore seems possible that, although the colour pattern of *virens* and *frutetorum* is inconspicuous as a whole, some detail in it will be sufficient to prevent mistaken attacks.

According to this view, the protection in *virens* and *frutetorum* against attacks by birds is based on the combined effects of unattractiveness and inconspicuousness. It is impossible, of course, to estimate which of the two is more important.

It is noteworthy that, within the group studied, attractiveness and conspicuousness are inversely related. Assuming that *pini* and *sertifer* have the same inferior attractiveness:

$$\xrightarrow{\text{attractiveness}} \qquad \xleftarrow{\text{conspicuousness}}$$

(pini + sertifer) *(nemoralis + simile)* *(virens + frutetorum)*

This binding suggests that the degree of conspicuousness which a relatively unattractive prey can permit itself decreases when the unattractiveness is lower. Hitherto, discussions in the literature are mainly restricted to the protective meaning of extreme conspicuousness combined with extreme unattractiveness (COTT, 1957). POULTON (1890) has introduced the term aposematic for the animals concerned and has argued that this combination must be of protective value by reducing the chance that, when detected by a bird, a potential prey is actually attacked, erroneously from the point of view of the bird but still harmful to the prey concerned. The data obtained on conspicuousness and unattractiveness show that it is justified to regard both *pini* and *sertifer*, and *nemoralis* and *simile* as aposematic[1]. However, animals with a more moderate degree of unattractiveness and conspicuousness exist as well, as shown above for *virens* and *frutetorum*, and it seems worth while to pay some attention to their protection in more detail.

COTT (1957), in his admirable account of the function of conspicuousness in unattractive animals, suggests that any prey with a certain degree of unattractiveness needs very conspicuous, advertising colours in order to prevent erroneous tastings by birds. I cannot completely agree with this view. Firstly, as I have stated already (chapter III), it implies an underestimation of the power of discrimination in birds. Secondly, I think that, although intense conspicuousness is surely of high selective value in extremely unattractive species, animals of a more moderate degree of unattractiveness will profit most by an equally moderate conspicuousness.

To justify this statement, I must first stress that the intensity of predation on a species depends not on its attractiveness alone, but also on the general availability of food. This was first shown by SWYNNERTON (1915, 1919), who found that birds after long deprivation of food will eat even very distasteful insects, but that they become more selective as their appetite decreases. Near the repletion point only the

[1] Moreover, these species have some characters, serving an easy recognition, in common. The U-bend occurs in all four, stretching, jerking and gregarious habits in both *pini* and *sertifer*. It is possible, therefore, that they also profit by Müllerian mimicry (COTT, 1957).

most attractive species are accepted. A striking instance of the same phenomenon was established in the field by TINBERGEN. Although the aposematic moth *Atolmia rubricollis* was very common in the Hulshorst pine woods in 1953, the tits scarcely fed it to their young. In contrast, during a food shortage in the summer of 1954, it was consumed in considerable numbers.

In view of these facts, it is a plausible assumption that a conspicuous pattern may be advantageous in years with plenty of food by acting as a recognition mark for unpalatability, but harmful in years of scarcity by increasing the chance that the animal is detected by a predator. I assume that conspicuousness is the result of a gradual evolution. Any intensification will have entailed an increase both of the advantages and the disadvantages. As long as the result was a net gain in survival value, conspicuousness will have gone on increasing. The point where it comes to a stop depends on the attractiveness of the species concerned. Extreme conspicuousness is profitable only for highly unattractive species, because they are practically never preyed upon. On the contrary, species with a more moderate degree of unattractiveness are in great danger of predation in years of food scarcity. This might be disastrous if the species were very conspicuous. Therefore, such species will not intensify their conspicuousness beyond a moderate degree. In a species that is only slightly unattractive, the danger of predation is so great, that a low level of conspicuousness is the most profitable.

In my opinion, the above is a promising explanation of the differences in conspicuousness among the sawfly larvae. It suggests that in both *virens* and *frutetorum* unattractiveness is too weak to permit the evolution of recognition marks as conspicuous as those of the less attractive species. The intermediate unattractiveness of *nemoralis* and *simile* is the reason why these, in turn, are less conspicuous than *pini* and *sertifer* which are most unattractive of all.

I think that, in view of the nature of food selection in important insectivorous birds such as tits, the existence of intergradations between conspicuousness and inconspicuousness in insects deserves serious consideration. Hitherto, this seems to have escaped attention. I have found only one approach to this aspect of adaptive coloration in the literature. MOTTRAM (1918) wrote: "If animals can be arranged in an order of palatability, and if warning coloration and protective resemblance are the result of selection by enemies, then animals liable to attack should form a series presenting all grades, from a most pronounced conspicuous coloration to a great protective resemblance, the intermediate forms showing something of both". Mottram does not discuss how such a gradation could have evolved. As a functional background,

he suggests only that differences in conspicuousness enable birds to diagnose the relative palatability of a prey at sight. Mottram has drawn this conclusion from an ecological investigation on fishes. In addition, his observations on hunting technique in this group show a basic similarity in this respect between fishes and tits (Tinbergen). It may well be, therefore, that widely different predators select their food in a similar manner, and that therefore gradations in conspicuousness will occur in many other classes of prey besides the insects.

I have found three papers in the literature in which an attempt is made to correlate conspicuousness and palatability of prey, but which do not permit a conclusive check of the view presented here.

Carpenter (1921) classified a great number of African insects according to conspicuousness to man, and tested their palatability to two monkeys. He found that of the highly conspicuous species 66 % was very distasteful, 34 % moderately distasteful or even palatable (percentages calculated by me from Carpenter's figures on pp. 101 and 103). However, Carpenter has not presented the insects on their natural backgrounds. This will have made difficult an exact evaluation of their conspicuousness in nature. Also it may be questioned whether monkeys contribute materially to the selection for colour pattern in African insects. Therefore his results cannot be used as evidence against our hypothesis.

Jones (1932, 1934), feeding insects on trays to wild birds, has found that the more attractive species are generally dull coloured, whereas species usually refused are gaudily attired with yellow, orange or red, or have a brilliant metallic colour. However, for the species of intermediate acceptability, which we would expect to be moderately conspicuous, Jones also records colours which seem to be highly conspicuous: white, black and white, pink and pale yellow. Unfortunately, no definite conclusion can be based on these data, because Jones' comparison of the coloration types is highly schematic. He only mentions the colours, and characterizes the patterns rather roughly as dull or bright, without giving exact descriptions of their effects with regard to the natural background.

Finally, Cott (1946) has estimated the relative conspicuousness and edibility of 38 species of African birds. He used hornets to test edibility, but, since he regarded this as a drawback himself, he made a number of tests with man and cat as a check. Cott has found a general tendency for conspicuousness to go up in this group of prey species when their edibility decreases, but he does not discuss the ecological background of this correlation. His main conclusion is that conspicuous colours, which, according to ethological findings, often function as intraspecific social releasers in birds, may also subserve an aposematic function, thus acting as interspecific social releasers.

I do not think it surprising, that so few papers relevant to our problem are to be found in the literature. The current view on coloration as a defence against predators accentuates too much the two extreme types: exclusively cryptic systems in attractive species, high conspicuousness in the unattractive ones. Such a view easily leads to the neglect of the study of gradations in both characters. I hope that the ideas presented above may help to direct more attention to these gradations in future, for this may elucidate, as a further elaboration of the theory of adaptive

coloration, that the degree of conspicuousness must be adjusted to the degree of unattractiveness.

VI. SUMMARY

This paper deals with the problem how some species of sawfly larvae (genera: *Diprion* and *Neodiprion*), which are scarcely preyed upon by titmice, are protected from birds. In addition, a possible protection against parasites has been investigated.

It has been found that an approaching bird (a Great Tit or a Chaffinch) or a parasite can release three types of display responses (for descriptions see chapter I) in the larvae studied. Two of these types, restricted to the gregarious species *Diprion pini* and *Neodiprion sertifer*, can intimidate a parasite or, temporarily, a bird. The conclusion has been drawn that the responsiveness of the gregarious larvae lowers the reproduction rate of *Exenterus*, an important hymenopterous parasite. In turn, the parasites studied (Ichneumonidae and Tachinidae) show adaptive behaviour to avoid releasing displays in their hosts. The small number of hymenopterous parasites adapted to the larval stage of the sawfly species and the relatively high number of species attacking the cocoons, may point to another way of *protection* against the responsiveness of the larvae, viz. avoidance of the larvae as hosts (chapter II).

To investigate how far detectability might be responsible for the low predation, a study was made of four characteristics contributing to the visual detectability of the larvae. Differences were found between the six species investigated. The larvae of *Diprion pini* and *Neodiprion sertifer* were found to be the most conspicuous of all species because of their gregariousness, their eye-catching displays, and their high readiness to respond to external stimuli; moreover, in *pini* conspicuousness is enhanced by a relatively large size and a very contrasting colour pattern. *Diprion nemoralis* and *Diprion simile* are of intermediate conspicuousness. Their responsiveness is also high, they are rather big, and they have a contrasting, non-disruptive coloration, but they are less eye-catching because they are solitary. The solitary species *Diprion virens* and *Diprion frutetorum* are considerably less conspicuous owing to the combined effects of a low responsiveness and a non-contrasting colour pattern, which, moreover, in *virens* is disruptive.

Another possible factor, preventing predation might be unattractiveness. This has been studied in feeding experiments with wild Great Tits and with captive Chaffinches (chapter IV). The highly conspicuous, gregarious species were found the least attractive. *Diprion nemoralis* and *simile*, intermediate as to conspicuousness, were also found to be of intermediate attractiveness. The relatively inconspicuous *Diprion*

virens and *Diprion frutetorum* were less unattractive but still not highly appreciated by the wild birds. The unattractiveness was in all species proved to be due to a resinous liquid from two diverticula from the foregut of the larvae.

In chapter v it has been concluded that the gregarious species *Diprion pini* and *Neodiprion sertifer* (apart from the temporary intimidating effect mentioned above), and the solitary species *Diprion nemoralis* and *Diprion simile* are protected from birds by unattractiveness in combination with a relatively high conspicuousness, which prevents erroneous attacks of experienced birds. It has been argued that *Diprion virens* and *Diprion frutetorum* can be recognized specifically by birds despite of their inconspicuousness. Consequently in years food is abundant they will enjoy protection against birds because of their relative unattractiveness; in years with scarcity of food birds will start to prey upon them but nevertheless predation will remain low because of their inconspicuousness.

To explain the selective advantage of the phenomenon that *pini* and *sertifer* are most conspicuous and least attractive, *virens* and *frutetorum* less conspicuous and most attractive, while *nemoralis* and *simile* are intermediate in both respects, it has been suggested that in relatively unattractive species of prey the degree of conspicuousness must be adjusted to the degree of unattractiveness, in order to prevent a disastrous predation in years with scarcity of food.

In chapter III it was shown that special behaviour patterns help the gregarious larvae to maintenain their colonies. The larvae are positively attracted by each other, absence of contact with other larvae leads to searching behaviour that enables a larva to catch up with the colony, scent trails are used as a means of communication when moving to a new feeding place.

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FURTHER EVIDENCE FOR THE ROLE OF "SEARCHING IMAGES" IN THE HUNTING BEHAVIOUR OF TITMICE

by

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I. INTRODUCTION

This study deals with some aspects of the relation of the Great Tit (*Parus major* L.) to one of its prey species, the moth of the Bordered White, *Bupalus piniarius* L. It was undertaken as a part of the work of Professor Dr. L. TINBERGEN on the ecological role of Titmice and other songbirds in pinewoods.

One of the most important points in TINBERGEN's analysis is the elucidation of the correlation between the density of a certain prey and the proportion of this prey in the Tit's food. He formulated a probability of encounter hypothesis which can be written as follows:

$$P_A = \frac{R_A \cdot D_A \cdot 100}{R_A \cdot D_A + R_O \cdot D_O} = \frac{D_A \cdot 100}{D_A + \dfrac{R_O \cdot D_O}{R_A}}$$

P_A = percentage of prey species A in the food.
D_A = density of prey species A in the territory.
R_A = risk index[1] of prey species A.
D_O = total density of all prey species other than A.
R_O = average risk index of a 'non – A' prey.

Graphs representing P_A as a function of D_A for given values of $R_O \cdot D_O / R_A$ will be called expectation curves, following TINBERGEN. The question arises whether the probability of encounter hypothesis is correct, *i.e.* whether the data collected in the field will fit such an expectation curve.

TINBERGEN found that if one has an expectation curve that fits the

[1] The risk index R is the proportionality factor which, on the probability of encounter hypothesis, relates the number of captures N to the density D and hunting time t, i.e., $N = RDt$ (TINBERGEN, in this volume).

observed values of P_A at intermediate densities, the percentages at both low and high densities fall below expectation. He argues that these discrepancies occur because R_A varies with D_A. He attributes the low risk at high prey densities to a tendency in the birds to keep their menu varied.

On the other hand he presents evidence that the risk remains low at low densities because the birds gain insufficient experience with the prey. With increasing density they learn to catch it more efficiently, and risk goes up.

Only for one species *(Acantholyda)* TINBERGEN had a sufficient number of data to test the reality of this learning process statistically, for tree living species have a serious drawback for work of this kind. The density measurements require a very elaborate sampling technique. As a consequence a census could be made twice a month only, resulting in a few pairs of corresponding values of P_A and D_A per season, for each species is available only during part of the season.

In 1950 L. TINBERGEN, D. VAN DER HEY, and N. PROP found that the moths of *Bupalus piniarius* are a good object for the study of the same problems. *Bupalus* moths rest on the forest floor until about 10 a.m. each morning during the last days of May and all of June. These moths are very vulnerable to predation by birds, and they form an important part of the food of Tit nestlings. It is relatively easy to census these moths, and when the composition of the food of nestlings is determined simultaneously, it is possible to obtain for each morning a pair of values for the density of moths on the ground and their proportion in the food.

After a discussion of our methods we shall consider in sections III and IV the elements of the behaviour of *Bupalus* moths and Great Tits that are relevant to our problem. It will be shown that the *Bupalus* moths fed to the nestlings during the morning hours, are the same as those censused on the forest floor. Thereafter we shall analyse the percentage density relation for *Bupalus* in much the same way as TINBERGEN has done for various prey species.

We shall discuss only the learning process (formation of a searching image in TINBERGEN's terminology). Our material contains no indication of a decrease of risk at high densities. This may be because Tits catch *Bupalus* moths almost exclusively in the morning. It is improbable that the tendency of a bird to keep its menu varied is distinct in such short periods.

The study was carried out in 1951–1955 under the supervision of and in collaboration with Professor Dr. L. TINBERGEN. It will be clear that we owe very much to his ideas, his guidance and his help.

With the *Bupalus* census and the determination of the composition of the food all our fellow students working in Hulshorst helped us.

Our thanks are extended also to Mr. P. VAN DER LAAN of the Institute for Theoretical Biology (director Professor Dr. H. R. VAN DER VAART) at Leiden, for the statistical evaluation of our data, and to Professor Dr. L. DE RUITER for stimulating discussions and help with the manuscript. Dr. D. M. DAVIES (Mc Master University, Hamilton, Canada) was so kind to help with the preparation of the English text.

II. METHODS

The investigation was carried out in the same wood, "De Zwarte Berg", in Hulshorst where TINBERGEN worked. For a description of the wood and for some of the methods used we can refer to his paper in this volume.

The food records were obtained each morning continuously from 7–10 a.m. The reason for choosing this period is given in the chapter on the behaviour of *Bupalus* moths. For each prey brought to the nest-box notes were made on sex, inflation of the wings, hardness or softness of the wings, "fresh" (emerged the same morning) or "old" (one day old or older) appearance of the moth, and on winglessness.

The census of emerging Bordered Whites was taken by counting the moths on areas of 10 m $\times$ 1 m on the forest floor. These census areas were marked out with cord and were situated in a circle with a radius of approx. 100 m around the observation hides. The number of census plots as a rule varied between 24 and 45. The plots were distributed over the territories of the Tits of which the food records were taken.

The procedure of a census was as follows: each plot was scrutinized for about 7 minutes during each of two periods: one from 7–8.30 and one from 8.30–10 a.m. Of all moths found during these inspections the sex and the state in which they were found was noted. Moths found during the first round were taken away, so that they could not be counted a second time.

III. THE BEHAVIOUR OF BUPALUS MOTHS

Bupalus piniarius hibernates as a pupa in the soil. The day on which the emergence starts differs from year to year, but in Hulshorst the first moths may be expected between May 20th and May 30th. Moths emerge only during the morning hours, mostly between 6 and 8.30 a.m. We found no newly emerged individuals after 8.30 a.m. The moths rest for some time after emergence, hanging from the underside of dead twigs and pine needles that lie on the ground. They stay thus until the wings are expanded and have become quite dry. Then they fly to the treetops, where they swarm around for a while and copulate, and where

all stay for most of the rest of their lives. The time at which this swarming occurs varies from day to day. It starts usually at about 10.30 a.m., but exceptionally as early as 9.15 a.m. On some days the moths start flying as late as 1 p.m., or remain on the ground for the whole day. This occurs mainly on cold, wet and rainy days. On such days there are also older moths from the treetops that have moved to the ground level and are found especially on dead twigs and branches. The moths are much less agile on these days than on sunny days and it is probable that they can be caught much more easily by the Tits. As will be seen later this influences the interpretation of the searching behaviour of the Great Tits.

IV. THE CAPTURE OF THE MOTHS BY TITS

In 1951 it became obvious that there exists a marked diurnal periodicity in the percentage of *Bupalus* moths in the food brought to the nestlings. Table I contains the averages of all data of 1951.

TABLE I

Diurnal Periodicity of % of *Bupalus* in the food in 1951

Time of day	% of *Bupalus* in the food
6.30– 9.30	22
9.30–12.30	14
12.30–16.30	2.5
16.30–19.30	2

The coincidence of the emergence of Bordered Whites with the peak in the *Bupalus* captures by the Tits led TINBERGEN to the tentative conclusion that the Tits hunt mainly for newly emerged moths on the ground, and that "old" moths in the crowns of the trees are taken only exceptionally.

In 1953 special attention was given to this problem. The periods of observation in the nestboxes were spaced evenly over the day, while in other years most of the observing was done between 7 and 10 a.m. It was found that the results varied from day to day according whether swarming of the moths was observed. The result for the Great Tit pairs 8 and 10 are shown in Table II.

In column 4 the ratio of the percentages is given, the percentage of *Bupalus* from 7–10 a.m. being unity. The percentages themselves are not comparable for the various days as the density of emerging moths has a very great influence on them.

TABLE II

Periodicity in the catching of *Bupalus* by Great Tit pairs 8 and 10 (1953)

Behaviour of the moths	Time	Total number of prey	Number of *Bupalus*	Percentage of *Bupalus*	Ratio of *Bupalus* percent. (7–10 = 1.00)
No swarming	7–10	264	74	28	1.00
May 30th, 31st	10–13	276	85	31	1.10
June 1st, 10th	13–16	398	128	32	1.14
	16–19	93	31	33	1.18
Swarming of only few of	7–10	466	294	63	1.00
the moths starting late	10–13	314	205	65	1.03
in the morning	13–16	115	37	32	0.51
June 3rd–5th	16–19	289	96	33	0.52
Swarming starting	7–10	635	213	34	1.00
between 9.15 and 11 a.m.	10–13	369	36	10	0.30
June 6th–9th	13–16	143	17	12	0.35
	16–19	237	14	6	0.18

The results, so far, indicate that the Bordered Whites caught by the Tits, are taken from the newly emerged *Bupalus* moths on the ground.

This is also the reason why these observations were usually restricted to the period from 7–10 a.m. In this period we could be sure that most of the moths had emerged before the start of the observations, and that the rising from the ground had no appreciable influence on the data.

One could argue, however, that the weather does not only influence the swarming of fresh *Bupalus*, but also the mobility of the old moths that are already in the tree tops. Then it might be that on dark and wet days the Tits take moths both from the tree tops and from the ground, as is suggested by the observation that on some wet and cold days of 1954 and 1955 a considerable portion of old moths were fed to the nestlings.

Now it is of the greatest importance to make sure from which layer(s) of the wood the *Bupalus* moths are taken by the Tits during the morning hours, for a comparison of food percentages with densities can be meaningful only if the density figures have been obtained in exactly the same layer(s). We have used two methods in our attempts to settle this question.

1. DIRECT OBSERVATION AT THE NESTBOX

During the early morning hours it can easily be seen that most of the *Bupalus* moths fed to the young must have emerged the same day. For

instance moths with only partially expanded wings or wings that are still soft are often seen. In most cases the observers have put down in their notes the condition in which the moths are brought to the box. It is not possible to decide in every instance if the moths brought are "fresh" or "old". Moths with dry wings can only tentatively be classified as "fresh" when the colours of the wings show exceptional freshness. Old individuals can sometimes be recognised from their weathered outlook, but with the many gradiations in between a decision is difficult. Moreover, the tits sometimes have removed the wings from a moth before feeding it to the young. This is done often by tits that have young nestlings.

On most days there is no indication that "old" *Bupalus* moths form an appreciable part of the total number of *Bupalus* moths fed. On some days in 1954 and 1955, however, many "old" moths were brought to the nestbox. We tried to determine their place of origin with the following method.

2. SIMULTANEOUS WATCHING AT THE NESTBOX AND IN A HIDE OUTSIDE

In 1952 and 1954 it was often possible to show that, at times when many *Bupalus* moths were brought to the nestbox, much ground-feeding was done by the tits.

In 1955 this problem was tackled more systematically. By noting the times of the visits of the parent tits both by an observer at the nestbox and by an observer in a hide approx. 50 m from the nestbox, it was hoped that, for each individual prey brought, it would be possible to determine from where it came. This proved to be impossible in most cases as the time readings were not quite exact, and as the observer in the hide outside could not come as near to the nestbox as to be able to determine the sex of the feeding Tit. Moreover, he often lost sight of one or both of the Tits while they were hunting.

On June 8th and 9th 1955 many "old" *Bupalus* moths were brought to the nestbox and on June 6th a good number. Analyzing the observations (of nestbox 4) of June 6th and 8th we find for some *Bupalus* moths, classified as "fresh", indications that they were taken from the treetops. Now this is virtually impossible and it shows the inadequacy of this method of coördinating observations. For such days, on which hunting in the treetops and on the ground for both Tits of a pair alternate very frequently, it was impossible to determine from the notes the layer where every individual prey was caught.

A stroke of luck provided us with the following data from June 9th. TINBERGEN, observing in the hide outside, saw that between 8.42 and 9.34 a.m. the two Tits of nestbox 4 were continuously hunting on the ground. During this period Miss G. DE VRIES counted a total of 55

Bupalus moths brought to the nestbox, and classified these as follows: 32 fresh, 17 old and 6 age unknown. During the whole morning the tits brought moths in the same ratio of fresh and old ones, so we can assume with reasonable certainty that all or nearly all of the *Bupalus* moths brought on June 9th came from the ground.

Here we have one day of which we know for certain that the old moths brought came from the ground. For other days we have indications that at least some of the old *Bupalus* moths were collected on the ground. The pairs 22 and 4 in 1955 did much ground-feeding during all days of the observation period, but we have no direct evidence that all or most of the moths brought were collected in the ground layer. The same applies to the observations from the days June 10th–15th, 1954. For these days we have no direct observations about the hunting of the Tits, but many old *Bupalus* moths were found resting on the ground in the census plots.

It seems to us justifiable to conclude that the bulk of the old moths brought to the nestbox during the early morning hours were collected on the forest floor. On account of this we have correlated the census data for all *Bupalus* moths on the ground with the percentage of all *Bupalus* moths in the food.

V. RESULTS AND DISCUSSION

We cannot show here all the graphs of the different sets of data. Examples of three types are given in Figures 1–3.

These graphs show the densities of *Bupalus* on the ground (D_B) on the abscissa, and the percentage of *Bupalus* moths in the food (P_B) on the ordinate. For each point the date of the observation is added (during the end of May and the beginning of June). In the graphs three expectation curves are given, for values of $R_O \cdot D_O / R_B$ suggested by the observed data. These values are given in the graphs next to the respective expectation curves.

Even in the most favourable case (fig. 1) it is clearly impossible to find any single expectation curve, that will fit all the points plotted. One possible source for the discrepancies will be random fluctuations in P_B and D_B. We have also to investigate the possibility of systematic deviations from expectation. TINBERGEN found in practically all of the prey species studied by him that the actual relationship departs from the expectation curves because at low densities the consumption is extra low. This phenomenon cannot be detected in figs. 1 and 3, but in fig. 2 it is obvious. We can see also in fig. 2 that the relatively low values of P_B belong to observations from the earlier days. After May 28th *Bupalus* consumption is much higher.

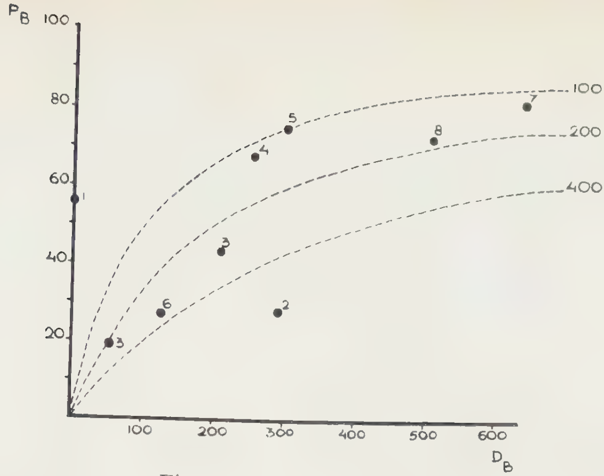

Fig. 1. G.T. 18 ♂ (1954).

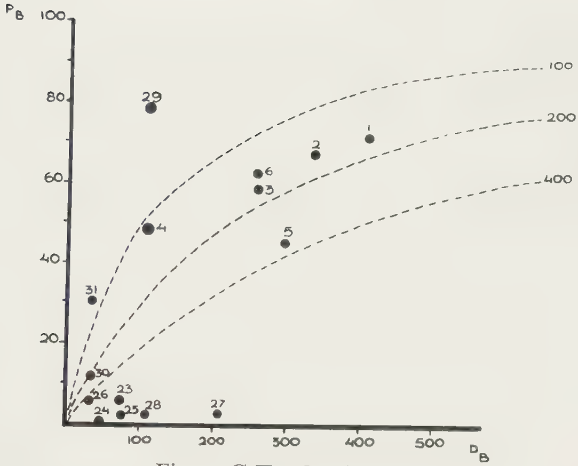

Fig. 2. G.T. 36 ♀ (1952).

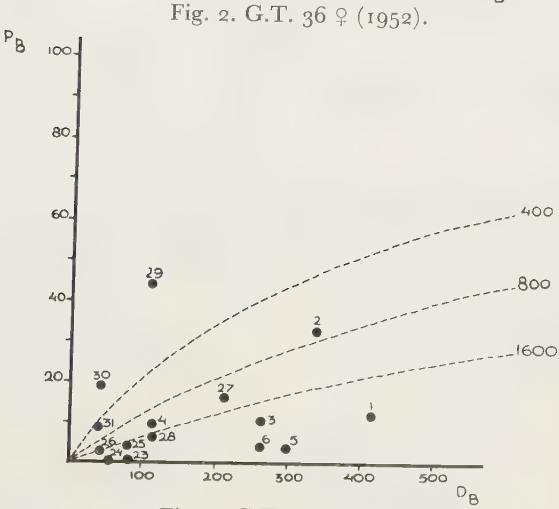

Fig. 3. G.T. 13 ♂ (1952).

Fig. 1–3. Graphs of the relation between *Bupalus* density and the percentage of *Bupalus* in the food. Explanation in the text.

We have asked ourselves if this low consumption (and therefore high values of $R_O \cdot D_O/R_B$) is a statistically significant phenomenon. To investigate this we have used a method which is to a great extent the same as Tinbergen used for his *Acantholyda* data. For each simultaneously determined set of P_B and D_B we have calculated an estimate $R_O \cdot D_O/R_B$ with the formula

$$\frac{R_O \cdot D_O}{R_B} = \frac{100 - P_B}{P_B} \cdot D_B = \frac{N - B}{B} \cdot D_B$$

N = total number of prey.
B = number of *Bupalus* fed.

We have the advantage that we are able to investigate the trend of $R_O \cdot D_O/R_B$ in time, which is a more direct way to detect learning processes than the correlation of $R_D \cdot D_O/R_A$ with D_A that TINBERGEN had to use. In tables III to VI we have summarized the results of all our observations.

The values of $D_B \cdot (N--B)/B$ in columns 6 of tables III–VI were tested for trend against time with a nonparametric test described by MANN (1945). This test can be summarized as follows:

With a series of n_k mutually independent variables: $x_1, x_2, \cdots x_{n_k}$, where the subscript denotes time, MANN defines the following statistic T. T counts the number of inequalities $x_i < x_j$, where $i < j$. For each pair (x_i, x_j) with $x_i = x_j$, a score of $\frac{1}{2}$ is added to T. When there is no trend the mean value of T will be $ET = n_k(n_k - 1)/4$. For a T_k found in a certain series we can calculate the probability α_k to find a value $T \leqslant T_k$, assuming that there is no trend (null-hypothesis). When we fix the level of significance at 0.05, then an $\alpha_k < 0.025$ means a significant downward trend, an α_k between 0.025 and 0.975 means no significant trend, and an $\alpha_k > 0.975$ means a significant upward trend.

In table VII the values of n_k, ET, T_k and α_k are given for each series of data of the individual birds. Out of a total of 16 birds 6 show a a significant downward trend, 1 shows a significant upward trend and 9 show no significant trend. The question arises what this result implies for an average individual. To this end we want to consider certain combinations of the results found. The significance of combined tests of significance can be tested by the method of PEARSON (1938). According to this theory $-2 \log_e Q = -2 \sum_{i=1}^{m} \log_e \alpha_k$ is distributed as chi-square with $f = 2$ m degrees of freedom, when the distribution of each individual α_k is rectangular over the interval (0.1). In fact, the

TABLE III

1952

Census data of *Bupalus* density and composition of food between 7 and 10 a.m.

	Emerging *Bupalus*			G.T. 13 male			G.T. 13 female			G.T. 36 male			G.T. 36 female		
	1	2	3	4	5	6	4	5	6	4	5	6	4	5	6
Date	a	b	D_B	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$
May 23	1	14	72							35	5	432	67	3	1,536
24	1	21	48							53	0	∞	68	0	∞
25	2	27	74	48	2	1,702	53	7	486	42	0	∞	51	1	3,700
26	1	27	37	47	1	1,702	68	1	2,479	37	3	419	56	3	654
27	6	27	222	50	8	1,165	43	1	9,324	38	1	8,214	47	1	10,212
28	3	27	111	60	4	1,554	88	18	432	35	0	∞	45	1	4,884
29	3	27	111	70	31	140	129	60	128	66	38	83	63	49	33
30	1	27	37	65	12	163	129	6	759	56	11	151	81	9	296
31	1	27	37	65	5	444	89	13	216	35	5	222	66	20	85
June 1	11	27	407	27	3	3,256	161	133	86	60	33	333	80	57	164
2	9	27	333	44	13	795	119	86	128	53	37	144	88	59	164
3	7	27	259	50	5	2,331	116	21	1,171	65	28	342	86	52	169
4	3	27	111	60	5	1,221	81	24	263	61	17	287	110	53	119
5	8	27	296	58	1	16,872	123	12	2,738	76	40	266	126	57	358
6	7	27	259	36	1	9,065	86	6	3,453	98	63	144	85	53	156

a = Number of *Bupalus* moths found.
b = Number of sampling plots (of 10 square meters each) examined.
D_B = Calculated number of *Bupalus* moths on 10,000 square meters.
N = Number of prey brought to nestbox between 7 and 10 a.m.
B = Number of *Bupalus* moths brought to nestbox between 7 and 10 a.m.

TABLE IV

1954 I en II

Census data of *Bupalus* density and composition of food between 7 and 10 a.m. Explanation of symbols see table III

	Emerging *Bupalus*			G.T. 29 male			G.T. 29 female			G.T. 18 male			G.T. 18 female		
	1	2	3	4	5	6	4	5	6	4	5	6	4	5	6
Date	a	b	D_B	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$
May 26	0	24	0	75	10	0	44	2	0						
27	3	24	125	82	17	478	42	9	458						
28	4	24	167	73	17	550	53	16	386						
29	2	24	83	85	35	118	67	17	244						
30	7	24	292	104	27	833	73	22	677						
31	1	24	42	74	4	735	85	8	404	68	13	178	48	2	966
June 1	0	24	0	91	14	0	33	4	0	54	30	0	83	11	0
2	7	24	292	126	37	702				102	29	735	182	11	4,539
3	5	24	208							109	47	274	143	8	3,510
4	6	24	250							123	84	116	121	42	470
5	7	24	292							116	87	97	147	52	533
6	3	24	125							117	32	332	195	16	1,398
7	15	24	625							158	130	135	197	42	2,307
8	18	36	500							105	78	173	106	63	341

TABLE V

1954 III

Census data of *Bupalus* density and composition of food between 7 and 10 a.m. Explanation of symbols see table III

	Emerging *Bupalus*			G.T. 33 male			G.T. 33 female			G.T. 38 male			G.T. 38 female		
	1	2	3	4	5	6	4	5	6	4	5	6	4	5	6
Date	a	b	D_B	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$
June 7	15	25	625	64	24	1,042	75	61	143	94	73	180	54	16	1,484
8	18	36	500	81	70	79	63	53	94	102	94	43	48	7	2,928
9	8	36	222	83	55	113	61	29	245	104	85	50	76	29	360
10	3	36	83	70	26	140	58	17	200	90	70	24	89	34	134
11	14	36	389	81	53	206	79	53	191	103	86	77	76	47	240
12	6	36	167	58	42	64	66	25	274	85	65	51	82	40	175
13	11	36	306	41	25	196	80	42	277	80	64	77	60	47	85
14	8	36	222	86	56	119	103	49	245	102	82	54	70	39	176
15	9	36	250	59	22	420	97	33	485	93	79	44	69	43	151

TABLE VI

1955

Census data of *Bupalus* density and composition of food between 7 and 10 a.m. Explanation of symbols see table III

	Emerging *Bupalus*			G.T. 4 male			G.T. 4 female			G.T. 22 male			G.T. 22 female		
	1	2	3	4	5	6	4	5	6	4	5	6	4	5	6
Date	a	b	D_B	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$	N	B	$\frac{N-B}{B} \cdot D_B$
May 30	2	45	44	26	1	1,125	17	0	∞	78	2	1,710	55	3	780
31	5	45	111	13	2	1,332	25	0	∞	126	1	13,875	52	1	5,661
June 1	4	45	89	27	2	1,113	35	0	∞	95	0	∞	55	1	4,806
2	7	45	156	30	2	2,184	27	3	1,248	79	1	12,168	57	2	4,290
3	4	45	89	41	2	1,736	61	1	5,340	72	0	∞	77	2	3,338
4	15	45	333	50	6	2,442	53	2	8,492	119	10	3,630	88	38	438
5	14	45	311	107	20	1,353	117	16	1,963	158	3	16,068	81	24	739
6	18	45	400	57	8	2,450	121	75	245	43	5	3,040	42	21	400
7	33	45	734	61	51	144	102	91	89	59	22	1,234	62	47	234
8	16	45	356	102	47	417	75	39	329	62	18	870	60	28	407
9	11	45	244	132	58	311	168	59	451	88	36	352	62	19	552
10	no samples taken														
11	no samples taken														
12	33	30	1,100	173	163	67	208	142	511	119	94	293	58	40	495

null-hypothesis of no trend implies that the distribution of each α_k is rectangular over (0.1). The alternative hypotheses are: the α_k's have shifted towards zero (in case of downward trend), and the α_k's have shifted towards one (in case of upward trend). In the first case the values of $-2 \log_e Q$ will tend to be large, in the second case these values will tend to be small. In table VII we listed as "Probability" the probability of finding a chi-square value larger than the value found for $-2 \log_e Q$. If these probabilities are small, this means that $-2 \log_e Q$ is significantly large, so that the conclusion of downward trend is warranted.

Although the result of the combination of all sixteen tests points to a downward trend ($-2 \log_e Q = 68.18$; $f = 32$; $P < 0.01$), there is, biologically speaking, more sense in combining data collected in more homogeneous circumstances, i.e. during the same period within a year. For this reason the data of 1952 and 1955 are treated as one group each and the data of 1954 are divided in three groups (see table VII).

The groups 1952 and 1955 show a significant downward trend against time. We cannot see in our data which of the factors of the complex $R_O \cdot D_O / R_B$ is responsible for this decrease with time, and we must look for other evidence bearing on this point.

TABLE VII

Results of statistical test of $\frac{R_O \cdot D_O}{R_B}$ against trend. For explanation see text

Year	Tit	n_k	ET	T_k	α_k	$-2 \log_e Q$	f	Probability
1952	♂ 13	13	39	49½	0.910	17.79	8	0.023
	♀ 13	13	39	40½	0.595			
	♂ 36	15	52½	30	0.015			
	♀ 36	15	52½	30½	0.017			
1954 I	♂ 29	8	14	17½	0.763	1.92	4	0.75
	♀ 29	7	10½	10½	0.500			
1954 II	♂ 18	9	18	18	0.500	2.93	4	0.57
	♀ 18	9	18	17	0.460			
1954 III	♂ 33	9	18	20	0.619	10.09	8	0.23
	♀ 33	9	18	29½	0.991			
	♂ 38	9	18	17½	0.480			
	♀ 38	9	18	8	0.022			
1955	♂ 4	12	33	24	0.123	34.33	8	< 0.001
	♀ 4	12	33	14½	0.007			
	♂ 22	12	33	13½	0.005			
	♀ 22	12	33	15	0.008			

Let us first consider the density of other food. D_O is always composed of the densities of a number of species: tree-living insect larvae and pupae, moths, spiders, pine seeds. Some of these will certainly decrease in numbers, e.g. by bird predation, or by reaching another developmental stage, which is not preyed upon by the birds. Others may increase in numbers. On the whole the density of prey available to the Tits is greater in mid June than it is in May (see TINBERGEN 1959, table III). There is no steady increase over this period, but it is likely that during the first two weeks that *Bupalus* moths emerge, the total prey density is at a minimum, until the time the caterpillar of *Panolis griseovariega ta*Goeze turns up in the food. On this basis the possibility cannot be excluded that the downward trend in $R_O \cdot D_O / R_B$ is due to a decrease in D_O.

However, it is evident from the columns *f* of tables III and VI that for the tits of pairs 36 in 1952, 4 and 22 in 1955 the shift from high values to low is very abrupt in 5 out of 6 individuals. It gives the impression of a switching from one level to another. Furthermore we call attention to the fact that the shift from higher to lower values in 1955 occurs on a different day in each series. It is unlikely that this sudden drop in the values of $R_O \cdot D_O / R_B$ could be caused by a drop in D_O.

Let us therefore turn to R_O. This quantity will change its value when the qualitative composition of D_O alters. These changes in R_O, however, will be small as R_O stands for a combination of prey, all of which have a much lower risk than *Bupalus* (TINBERGEN, 1959, table VII). The only cause for a sudden drop in R_O could be the disappearance of a favourite prey species. This is certainly not the case.

There are only two species other than *Bupalus* that have a high risk, viz. the caterpillars of *Dendrolimus pini* L. and *Panolis griseovariegata*.

The first mentioned is always very scarce and therefore cannot have a great influence. *Panolis* is more or less numerous in all years. *Panolis* larvae, however, turn up later in the food of the Tits than *Bupalus* moths, so that *Panolis* cannot give a drop in R_O.

The most likely explanation for the drop in the value of $R_O \cdot D_O / R_B$ in the observations of groups 1952 and 1955 is a change in the attitude of the Tits to *Bupalus*, that resulted in a great rise in the risk of, and thus in the predation on, *Bupalus*.

We come now to the questions of why the observations of 1952 and 1955 show an increase in the risk of *Bupalus* with time and of why those of 1954 (I, II and III) do not show this trend. It is easily seen that the observations of the series 1952 and 1955 started right at the beginning of the emergence period of *Bupalus*. This is not the case with 1954 (II and III). *Bupalus* moths had already been available to the Tits of

these groups for 4 and 12 days, respectively, when observations began. Why the two Tits of group 1954 (1) show no trend remains uncertain.

Casual observations done on earlier days, however, show that *Bupalus* moths were brought to nestbox 29 before May 26th, the first observation day. The ♂ 29 brings 5 *Bupalus* moths out of 39 prey on May 24th and 4 *Bupalus* out of 31 prey in the afternoon of May 25th. The comparable data of ♀ 29 are: May 24th, 0 *Bupalus*, total number of prey 29; May 25th, 3 *Bupalus*, total number of prey 20. As we have no census data for these days we cannot draw any definite conclusion about this, but there is an indication that these two birds may have completed their learning at a very early date.

The conclusion seems justified, therefore, that probably every year the risk of *Bupalus* moths is low in the first few days of the Bordered White season, but that it increases rapidly thereafter.

It has already been said that the cause of the rise in the risk of *Bupalus* should be sought in a change in the attitude of the Tits to these moths. One gets the impression that, after the Tits have had a certain number of contacts with the moths, they alter their searching behaviour in a way more directed to *Bupalus*. This is a learning process and the data show that this learning process can cause a more or less sudden change in the searching behaviour, because we mostly see a sudden rise in the relative consumption of *Bupalus*.

It would be possible that the difference in the searching method consisted mainly of a change from hunting in the tree tops to hunting on the ground. This is certainly an important factor but the data from nestbox 4, 1955 (table IX) show that it is not the only factor involved. As has been said before, simultaneous observations were done at the nestbox and at approximately 50 metres from the nestbox (to see where the Tits hunted). The duration of these observations was about 1 ½ hours between 7 and 10 a.m. on several mornings.

TABLE IX

Hunting Behaviour of Tits of Pair 4, 1955

Date	N	G	$\frac{G}{N}$	B	D_B	$\frac{G-B}{B} \cdot D_B$
June 3rd	56	17	0.30	3	89	416
4th	69	26	0.38	7	333	905
6th	65	50	0.77	34	400	188
7th	85	78	0.92	77	734	10
8th	88	44	0.50	31	356	149
9th	173	152	0.88	90	244	168

N = total number of feedings.
G = number of feedings after ground hunting.
B – number of *Bupalus* fed.
D_B = density of *Bupalus* on the ground (number per 10,000 sq.m.).

In table IX the rate of ground-hunting to total hunting, G/N is given. The last column shows estimates of the relative risk (R_G/R_B) multiplied by the density (D_G) of prey species on the ground other than *Bupalus*, according to the formula:

$$\frac{R_G}{R_B} \cdot D_G = \frac{G - B}{B} \cdot D_B$$

(*Cf.* the formula on p. 456).
R_G = risk index of non-*Bupalus* prey on the ground.
D_G = density of non-*Bupalus* prey on the ground.

In the same way as was done before, we can argue that the drop in (R_G/R_B). D_G is caused by a rise in R_B.

In other words, we see that after June 5th, there is an increase both in the proportion of ground hunting, and in the share of the Tit's attention allotted to *Bupalus* while the bird is on the ground. It is probable, therefore, that the Tits, when hunting *Bupalus*, use a searching image in the way described by TINBERGEN for other species of prey.

Our observations on *Bupalus* are in good agreement with TINBERGEN's concepts. TINBERGEN found a relatively low risk of a prey at low densities and argued that this was caused by a lack of experience in the birds. We have approached the question from another angle, looking for a change of risk of *Bupalus* in the course of time. We have found that when the Tits have no experience with the moths the risk of the moths is low. With increasing time there is an increase in the risk of *Bupalus*. We cannot say whether the rate at which the Tits have contacts with *Bupalus* must exceed a certain minimum for this learning process to occur, for *Bupalus* is always a more or less numerous prey.

VI. SUMMARY

In connection with the work of Prof. Dr. L. TINBERGEN on the role of songbirds in pinewoods the relations between the Great Tit, *Parus major*, as a predator and one of its preys, the moth *Bupalus piniarius*, were investigated.

Bupalus hibernates as a pupa in the soil. During the last week of May and the whole month of June emergence of the moths occurs, but only during the early morning. After emergence the moths rest for some time on the forest floor. Great Tits feed many of these moths to their young. There is good evidence that practically all *Bupalus* moths fed to the young during the morning hours are collected on the ground.

The density of *Bupalus* moths on the ground, and the percentage of *Bupalus* in the food a Tit takes to its nestlings, were established on certain days between 7 and 10 a.m. in 1952, 1954 and 1955.

For some Tits a statistically significant increase from low to high predation on *Bupalus* was apparent. These series commenced at the end of May when the emergence of *Bupalus* began. In the series of observations starting after *Bupalus* had already been available to the Tits for some time, no increase in the predation occurred; predation was high already.

The increase in the *Bupalus* consumption after the birds have been in contact with *Bupalus* for only a few days is attributed to a learning process in the Tits. These findings are in complete agreement with the results of the investigations of Tinbergen on other prey-species.

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FACTORS GOVERNING DENSITY IN THE CHAFFINCH (FRINGILLA COELEBS) IN DIFFERENT TYPES OF WOOD

by

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I. INTRODUCTION

Kluyver and Tinbergen (1953) have studied the regulation of the density of spring populations of titmice, particularly of the Great Tit, in the nesting area in woodland districts in the Netherlands. In the mixed woods densities of tit populations are high, in pine woods low. Therefore, the first type of wood seemed to be more attractive to the birds than the second. The fluctuations in density of spring populations from year to year are considerably smaller in mixed woods than in pine woods. An increase in density of the total spring population in an area leads to a very marked effect in pine woods, but is hardly reflected in the density in mixed woods. This reveals the existence of some buffer mechanism preventing the increase of the population density in the attractive mixed woods over a certain value. When in a given year the total population is greater than the mixed woods can accommodate, the surplus settles in the pine woods. This buffer effect was shown to depend on a balance between the attractiveness of the mixed woods and the repelling influence of fellow members of the same species defending their territories.

The present investigation, undertaken at the suggestion of Prof. Dr. L. Tinbergen, demonstrates a similar buffer mechanism in the chaffinch (*Fringilla coelebs* L.). The observations were made in the environment of the village Hulshorst near Harderwijk, by Tinbergen and his students. A short description of this district has been given by Kluyver and Tinbergen (1953). The woods in this area consist mainly of Scots Pine. However, on several places narrow strips of mixed wood, usually with a poor undergrowth and often on the borders of a small brook, are situated in the middle of these pine woods.

The population density is determined by counting and mapping the number of males advertising and defending a territory, particularly during the early hours after sunrise. In addition to the main observations carried out in spring I also looked for territorial activity in autumn, partly in a park-like mixed wood (140 ha) and in a pine wood with much undergrowth (100 ha) near Groningen (1951), partly in the Hulshorst district (1952).

II. CENSUS DATA

The density data for mixed wood and pine wood in the Hulshorst district are represented in figure 1. The density in the mixed woods studied is always considerably greater than that in the pine woods. The average for the top values in pine wood (1951, 1952 and 1953) is 3.5 males per 10 ha, to be compared with an average over the same years in mixed wood of 7.6 males per 10 ha. This means that chaffinches like titmice, also prefer mixed wood.

	Mixed Wood		Pine Wood	
	♂♂ on 25,5 HA	AVERAGE PER 10 H.A.	♂♂ on 72,8 H.A.	AVERAGE PER 10 H.A.
1946	19	7,2	8½	1,2
1947	17½	6,6	15	2,1
1948	23	8,7	15½	2,1
1949	17½	6,6	17½	2,4
1950	21½	8,1	20	2,7
1951	21	7,5	25	3,4
1952	19	7,2	26	3,6
1953	21½	8,1	24½	3,4
1954	18½	7,0	21	2,9
1955	17½	6,6	16½	2,3

Fig. 1. Fluctuation of the density of spring populations of the chaffinch in two types of wood during ten successive years.

The density of chaffinches in the pine woods shows a gradual increase from 1946 up to 1951, from 1951 to 1953 it is almost constant, after this a decline sets in. In contrast the density in the mixed woods during the whole observation period from 1946 up to 1955 shows

only small fluctuations oscillating around a constant level. The temporal pattern in the fluctuations is distinctly different in both types of wood.

In figure 2 I have plotted for the different years the number of territorial males in pine wood against those in mixed wood. This figure clearly shows the difference in size of the fluctuations in mixed wood and pine wood.

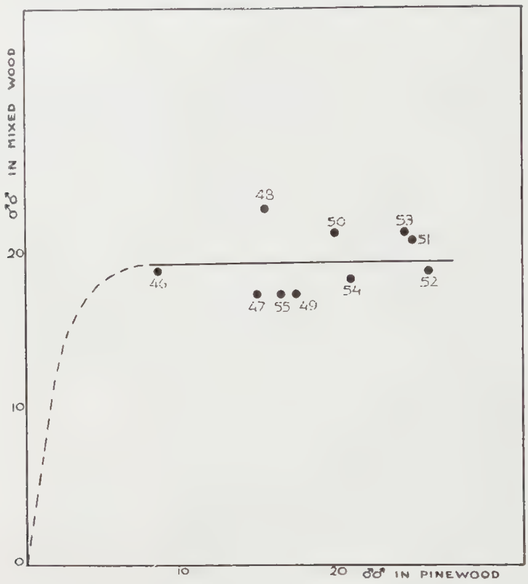

Fig. 2. A comparison of the number of territorial males in mixed wood and in pine wood in the different years of observation.

Because the mixed wood constitutes only a small part of the total wood area we may take the densities of the pine wood as indicative for the densities in the whole district. This means that our data show clearly that the population density in mixed wood is not influenced by the variations in size of the total population within the period studied. Even when the population was at its peak (1952), the density in the mixed woods did not increase over the average value, and the surplus of the population moved into the pine woods.

An important argument that birds settling in the pine woods do this only after having been repelled by the birds in the mixed woods, I obtained by following the course of the settlement in both types of wood during the early part of the season. In 1955 I counted the number of territorial males, in the Hulshorst area, in mixed woods

and in pine woods, the surfaces of which I chose—on the basis of the census in foregoing years—in such proportions that I could expect to encounter the same number of males in both types of wood.

The results have been plotted in figures 3 and 4. Figure 3 shows that the chaffinches started to settle in the pine woods at least thirteen days later than in the mixed woods. During the whole period of settlement the population in mixed wood was always higher than that in pine wood. In figure 4 for each separate day the number of territorial males in mixed wood is plotted against that found in pine wood (because actually the observations could not be carried out on the same day in both woods, the corresponding data have been derived from interpolation). The curve clearly shows the shift towards the pine wood when the density limit in the mixed wood is approached.

III. THE MECHANISM OF THE BUFFER EFFECT

The suggestion that the difference in density between chaffinch populations in mixed wood and in pine wood is caused by the interaction of a preference for mixed wood and a repellent effect of territory holding birds, means that actually habitat selection is responsible for the buffer effect.

However, before we adopt this conclusion we have to consider whether quite different causes might not account for this effect. We have to make sure that the populations of the chaffinches in both types of wood are not entirely independent, with their own rates of reproduction and mortality. Among other things we have to ascertain that the territories are not already established a long time before the reproductive season starts, because in that case differences in mortality during autumn and winter in the different habitats might have led to the different population densities in these habitats in spring. A short survey of the life-history of the chaffinch in the Netherlands will help to answer these questions. According to the bird-banding results the Dutch chaffinch can be considered a non-migratory bird (TINBERGEN, 1941, p. 43). At the end of the breeding season the finches leave the woods and during the day are usually found in flocks on the borders of open fields, particularly where cover is present. In autumn and winter they spend the nights in woodland, often in the outskirts, but they also penetrate deeper into the woods. A possibility exists that then chaffinches from mixed woods and chaffinches from pine woods each spend the night in their own habitat. However, there are no indications that this might be true, nor that such differences in roosting habitat might lead to differences in mortality.

Although I purposively looked for indications of territoriality in

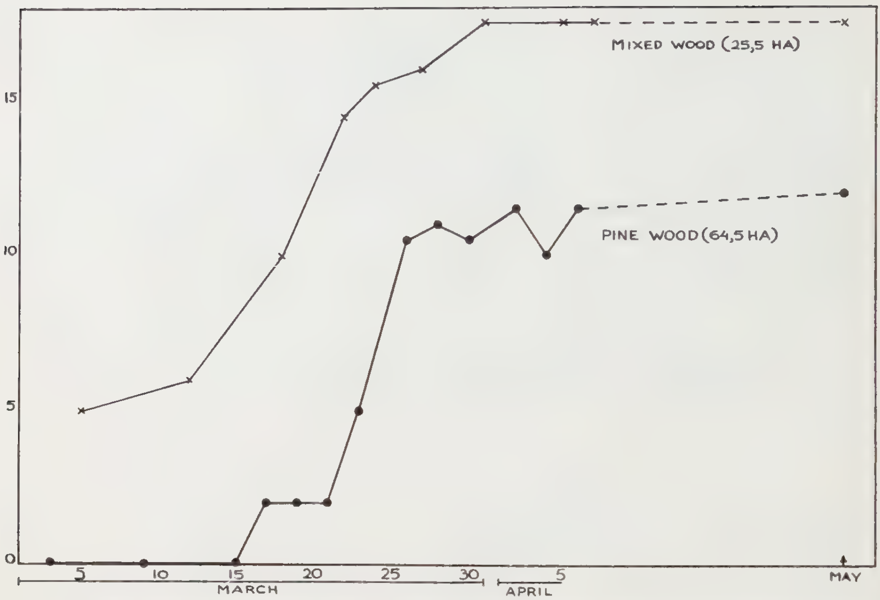

Fig. 3. The course of the settlement of male chaffinches in both types of wood in the spring of 1955.

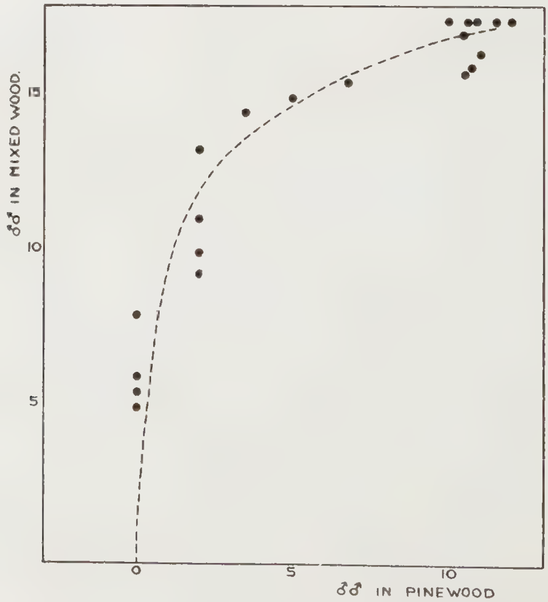

Fig. 4. A comparison of the number of territorial males in mixed wood and in pine wood in the course of the spring of 1955.

the chaffinches in autumn, I never found any. Settling in the territories occurs only in spring, well before home-migration of the Scandinavian population starts.

There is no indication that birds of the different types of wood remain separate outside the breeding season, therefore, it seems unlikely to me that differences in mortality outside the breeding season can account for the differences in density found in mixed woods and pine woods.

In the Hulshorst area the mixed woods are always closely connected with the pine woods. Therefore, independence of the populations in both types of wood seems only possible if the adult birds and their offspring would keep very strictly to the place where they have been born. Unfortunately we have no information on home tenacity in the Dutch chaffinches. BERGMAN (1953) reports that Finnish chaffinches have a strong tendency to keep to their territories, but he also mentioned several displacements over great distances. He has some indications that males stick more to the original territory than females.

From the present information it seems unlikely to me that entirely separate populations inhabit the mixed woods and the pine woods, and that their rate of reproduction and mortality is as markedly different as it would have to be to account for the considerable density differences shown by our census data. Consequently we conclude that these differences must depend on habitat selection, but in this habitat selection two factors play a role: the preference for mixed woods over pine woods, and the avoidance of areas strongly defended by other chaffinches of the same sex. This interpretation of the above mechanism implies that territories have a minimum size beyond which the aggressiveness of the defender becomes so high, that it is impossible for new birds to intrude effectively. Consequently we would expect territories in a mixed wood to have shrunken to this lower limit and therefore to be smaller than the territories in the pine woods. In fact even in the top years 1951 to 1953 the smallest territory in the pine woods was on the average about two times as large (2.64 ha) as that in the mixed woods (1.27 ha).

IV. SUMMARY

During a number of years the density of the spring populations of chaffinches in mixed woods and pine woods was determined and in one year the course of settlement in both types of wood was compared.

Whereas the densities in the mixed woods remained stable, that in the pine woods fluctuated markedly. Following KLUYVER and TINBERGEN, (1953) the buffering of density in the mixed woods is ex-

plained through habitat selection: it is argued that the chaffinches have a preference for mixed woods, but that some, because of the incompressibility of territories beyond a certain limit, have to move into the less attractive pine woods, when the mixed woods are fully occupied.

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TABLE DES MATIÈRES

L'ÉLECTROCARDIOLOGIE DU COEUR DE LA LAMPROIE

par

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I. INTRODUCTION

Ces recherches ont été entreprises à la suite de la publication d'Oets (1950) qui avait enregistré des électrocardiogrammes (ECG) de différents poissons. Oets a pu dériver chez les Anguilles *(Anguilla vulgaris)* à l'aide d'électrodes placées sur la peau, un tracé permettant de distinguer très nettement les différentes ondes. En fixant les électrodes sur la peau des Lamproies *(Petromyzon)*, Oets n'a pu obtenir un ECG que dans les cas où une des électrodes était placée au-dessus de l'ouverture ventrale du péricarde cartilagineux (Oets parle d'une fenêtre dans le péricarde). Dans ces cas aussi, les oscillations de l'ECG étaient extrêmement petites.

Ainsi qu'il ressort des travaux fondamentaux de Von Skramlik (1935, 1938), l'activité électrique du cœur des poissons et particulièrement des Cyclostomes, n'a guère encore fait l'objet de recherches. Zwaardemaker et Noyons (1910) ont été les premiers qui ont enregistré l'activité électrique des ventricules isolés d'Anguilles. Plus tard, bakker (1913) a dérivé les potentiels d'action des diverses parties du cœur chez les Anguilles. l'ECG du cœur isolé de Sélaciens a été étudié par de Meyer (1910), Hoffmann (1911), Arvanitaki et Cardot (1934).

Gitter (1933) a dérivé l'ECG d'Anguilles avec des électrodes, placées sur la peau. Il a étudié les fonctions du cœur et de la respiration des poissons intacts. Dans ces cas, les oscillations de l'ECG étaient fort petites. Oets (1950) a obtenu de meilleurs résultats; il a enregistré de distincts tracés chez des Anguilles intactes.

L'activité électrique du coeur des Cyclostomes, notamment des Lamproies, n'a été enregistrée que par Zwaardemaker (1922). En perfusant le cœur isolé avec du liquide de Ringer, généralement utilisé pour perfuser le cœur de Grenouilles, il a obtenu des ECG pratiquement analogues à ceux de la Grenouille.

II. MÉTHODIQUE

Les expériences ont été faites sur des Lamproies adultes *(Petromyzon fluviatilis)*. Pour l'enregistrement de l'ECG, on a utilisé un appareil électrocardiographique du type 'Elema Mingograph', dont la sensibilité pouvait être variée. Sur les courbes sont indiqués les étalonnages.

La dérivation des courants cardiaques a été effectuée à l'aide d'électrodes chlorées en argent ayant la forme de petites plaques ou de minces fils élastiques. Pour éviter l'influence des courants alternatifs, la préparation a été placée dans une cage de Faraday.

Pour la narcose, on s'est servi de l'uréthane; dans un certain nombre d'expériences, le cerveau et la moelle épinière ont été détruits afin d'immobiliser les Lamproies. Contrairement aux observations faites par VON SKRAMLIK dans ses expériences avec des poissons, l'hémorraghie provoquée par la destruction du système nerveux central des Lamproies, était relativement peu abondante. La respiration fut entretenu par un courant d'eau dirigé dans la cavité branchiale à l'aide d'une canule placée dans la bouche.

Nous avons dérivé les potentiels d'action du cœur aussi bien des Lamproies intactes que directement du cœur in situ. Le cœur de la Lamproie est enfermé dans un péricarde cartilagineux qui l'entoure comme une boîte solide. Le cœur est attaché au péricarde par quelques fils élastiques (AUGUSTINSSON, 1956). La partie antérieure du péricarde repose contre la cavité branchiale et sa paroi y est moins épaisse. Les vaisseaux sanguins percent le péricarde.

Pour isoler le cœur, la paroi ventrale a été ouverte à la ligne médiane; ensuite ont été enlevés les muscles adjacents, après avoir été libérés autour du péricarde. La partie ventrale du pericarde cartilagineux a été enlevée. Dans les expériences de perfusion du cœur avec le liquide de Ringer une canule de verre avait été insérée dans la veine jugulaire ou le sinus venosus; les autres veines ont été ligaturées. Pour certaines expériences, le cœur avait été enlevé du corps, fixé à une canule de Symes et perfusé d'un liquide de Ringer dont la teneur en potassium été légèrement réduite. J. B. ZWAARDEMAKER a également employé avec succès un Ringer de Grenouille pour la perfusion du cœur des Lamproies.

Pour l'orientation Mr. VAN GULIK a effectué dans notre laboratoire des déterminations de la teneur en électrolytes du sang des Lamproies à l'aide du photomètre à flamme. Il put constater que la teneur du sang en Na, K et Ca est du même ordre de grandeur que chez les Grenouilles. Ceci explique pourqoui les cœurs des Lamproies, isolés du corps et perfusés avec du liquide de Ringer de Grenouilles ont pu continuer de battre régulièrement pendant quelques heures.

III. DÉRIVATION DES COURANTS CARDIAQUES CHEZ DES LAMPROIES INTACTES

Les expériences relatives à la dérivation de l'ECG chez des Lamproies intactes à l'aide d'électrodes fixées sur la peau, n'ont pas donné de résultats satisfaisants. En fixant les électrodes des deux côtés du corps à hauteur du cœur (fig. 1) ou sur la surface ventrale juste au-dessus du cœur, nous avons obtenu des courbes présentant de très petits oscillations qui correspondent aux ondes de l'ECG des autres animaux. En plaçant les deux électrodes sur la surface ventrale, $\pm$ ½ cm. crânialement ou caudalement du cœur, on n'enregistrait généralement pas des courants cardiaques ou bien les oscillations de l'ECG étaient minimes. L'élimination du mucus cutané qui, selon OETS, augmente considérablement la résistance de la peau n'a donné aucune amélior ation.

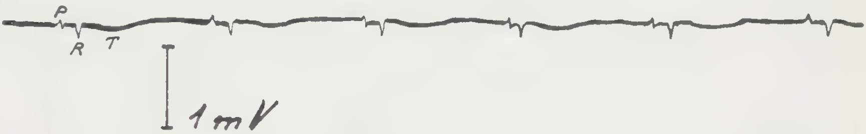

Fig. 1. Electrogramme dérivé de la peau au niveau du cœur.

Puisque la dérivation de l'ECG en fixant les électrodes sur la peau donnait des résultats insuffisants nous avons essayé de fixer les électrodes sur les muscles après avoir enlevé la peau. Au cours d'autres expériences, nous avons introduit, à travers la peau de l'animal intact, des électrodes en forme d'aiguilles dans la couche musculaire. Mais dans ces cas aussi les oscillation de l'ECG restaient fort petites.

Nous avons exécuté encore une série d'expériences dans lesquelles l'ECG était dérivé de la surface du péricarde cartilagineux. Cette méthode d'enregistrement où les deux électrodes étaient donc fixées sur le péricarde, a permis d'obtenir un tracé qui faisait ressortir plus ou moins nettement l'onde "P" et le complexe ventriculaire "QRS-T" (fig. 2). Après l'ouverture du péricarde des ECG analogues ont été obtenus.

Nous sommes parvenus aux mêmes résultats en déterminant les variations de potentiel électrique du cœur isolé qui était perfusé avec une

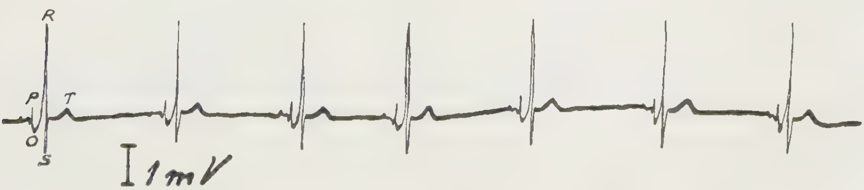

Fig. 2. ECG dérivé du péricard.

solution de Ringer. Les ECG obtenus de cette manière permettaient d'enregistrer toutes les ondes qui correspondaient à l'activité de l'oreillette et du ventricule.

Ces expériences permettent de constater que la dérivation de l'ECG à partir du péricarde cartilagineux, aussi bien lorsqu'il reste fermé qu'après avoir été ouvert, permet – avec l'appareil que nous avons utilisé – d'enregistrer des tracés où se retrouvent toutes les ondes qui ont été décrites pour les Anguilles et d'autres poissons et que J. B. Zwaardemaker a découvert également chez la Lamproie. Contrairement à la supposition d'Oets que la grande résistance du péricarde cartilagineux empêcherait précisement la dérivation de l'ECG à partir de la peau, nous avons pu obtenir des ECG de forme et de grandeur plus ou moins normales en dérivant de la surface du péricarde.

Des déterminations que nous avons entrepris pour orientation à l'aide du "Pont de mesure de Philips" (basé sur le principe du "Pont de Wheatstone") ont montré que la résistance électrique de la peau, des muscles, du péricarde et du cœur, ne donnent pas des valeurs différentes pour le tissu du péricarde cartilagineux. La résistance de tissus est à peu près la même que chez la Grenouille.

D'accord avec Einthoven (1908) et Samoiloff (1910) qui ont signalé la grande variabilité de la forme de l'ECG chez les Grenouilles nous avons pu constater que la forme de l'ECG peut varier assez fortement chez la Lamproie. Le mode de dérivation y joue aussi un grand rôle.

Il nous faut souligner une particularité du cœur de Lamproie. Dans les expériences avec la dérivation de potentiel d'action du cœur à l'aide d'électrodes fixées sur la peau ou sur les muscles nous n'avons pu obtenir des tracés présentants des ondes nettes. Ceci implique que les potentiels électriques provoqués dans le cœur ne peuvent être transmis jusqu'à la surface de la couche musculaire et de la peau qui se trouvent pourtant à une distance relativement peu éloignée. Ceci peut s'expliquer probablement par le fait que le champ électrique du cœur de la Lamproie est trop faible pour parcourir une certaine distance. Ce même phénomène se manifeste chez la Grenouille.

En étudiant la littérature sur l'activité électrique du cœur de la Grenouille, on est frappé par le fait que la plupart des expériences ont été faites avec des cœurs isolés et que l'on n'a guère procédé à des expériences avec un cœur en place, donc en fixant les électrodes sur la peau. Seuls les premiers investigateurs de l'activité électrique du cœur qui se sont occupés de ce problème après la construction du galvanomètre à corde par Einthoven, ont tâché de déterminer l'ECG de la Grenouille à partir de la peau. Aussi bien Samoiloff (1910) que Straub (1910) ont pu se convaincre que la dérivation des courants d'action du

cœur des Grenouilles intactes – donc à partir de la peau – donne des déviations de la corde minimes, qu'un enregistrement est généralement impossible.

EINTHOVEN (1908) lui-même avait déjà signalé que le cœur de la Grenouille se prête beaucoup moins pour l'enrégistrement de l'ECG que celui des Mammifères. Il n'a pu obtenir, lui aussi, un ECG convenable que par la dérivation directe de la surface du cœur mise à nu.

Nous avons mentionné quelque peu en détail ces anciennes expériences parce que nous nous sommes heurtés à des difficultés analogues dans nos propres expériences sur le cœur de Lamproie. Dans sa publication sur la physiologie du cœur des Poissons, VON SKRAMLIK (1935) signale, lui aussi, les mêmes particularités de l'activité électrique du cœur de ces animaux.

Si OETS put obtenir dans ses expériences des ECG typiques de la surface de la peau d'Anguilles, ce fait doit probablement être attribué à la grandeur du cœur; le cœur de l'Anguille étant relativement plus grand que celui de la Lamproie. D'ailleurs, le myocarde est bien plus développé aussi chez l'Anguille.

La position des électrodes sur le cœur isolé influence également la forme de l'ECG. Le fait a déjà été signalé par EINTHOVEN ET SAMOILOFF pour le cœur des Grenouilles et, plus récemment, par VON SKRAMLIK pour les cœurs des Poissons. Nos expériences avec les Lamproies nous ont permis de nous en convaincre.

Lorsqu'une électrode est fixée sur le sillon atrio-ventriculaire et l'autre sur l'apex du ventricule, on obtient des courbes dans lesquelles les ondes correspondantes à l'oreillette sont très petites ou manquent totalement. Le même fait a été noté par SAMOILOFF pour le cœur des Grenouilles. D'autre part, on trouve sur les tracés dérivés de l'oreillette, des ondes relativement petites qui correspondent à l'activité du ventricule. Ces faits démontrent que les potentiels d'action de l'oreillette sont trop faibles pour pouvoir être communiqués aux électrodes fixées sur le ventricule. Il en est de même, mais à un degré moindre pour les potentiels d'action du ventricule.

Par d'autres expériences nous avons essayé de déterminer les variations de potentiel électrique des diverses parties isolées du cœur.

IV. ACTIVITÉ ÉLECTRIQUE DU VENTRICULE

Lorsqu'on fixe les deux électrodes sur le ventricule à une certaine distance de l'oreillette, on peut dériver des courbes dans lesquelles on ne trouve que des ondes correspondantes à l'activité électrique du ventricule.

La fig. 3. montre les potentiels électriques du ventricule in situ, les

deux électrodes étant fixées sur la surface de l'organe. Cette courbe permet de distinguer deux ondes; l'une rapide, que l'on peut considérer comme l'onde "R" du ventricule et l'autre, beaucoup plus lente

Fig. 3. Electrogramme du ventricule.

qui vient à la suite de l'onde rapide et que l'on peut qualifier de "T" diphasique.

Sous l'influence de diverses manipulations, l'activité électrique du ventricule peut présenter des grandes altérations ce qui rend parfois difficile l'explication des courbes obtenues.

V. ACTIVITÉ ÉLECTRIQUE DE L'OREILLETTE

Dans une série d'expériences nous avons essayé d'enregistrer les potentiels d'action de l'oreillette. Le cœur avait été laissé en place et le ventricule séparé par un coup des ciseaux bien aiguisés. Ceci ne présente pas de difficulté étant donné que l'oreillette et le ventricule ne sont reliés que par un isthme étroit. Après cette opération, le sinus et l'oreillette continuaient à battre. Néanmoins, une dissociation se manifestait toujours dans le fonctionnement de ces parties du cœur: dans la plupart des cas, 4 à 5 contractions du sinus étaient suivies d'une seule contraction de l'oreillette. Ce bloc sino-auriculaire doit être attribué à la lésion inévitable de l'oreillette pendant sa séparation du ventricule.

L'opération du cœur entraîne, outre le bloc sino-auriculaire, une modification dans la forme des variations de potentiel électrique de l'oreillette. Au lieu d'un sommet "P", typique pour l'ECG normal, on a enregistré une activité électrique compliquée. On pouvait distinguer 2 ou 3 ondes diphasiques, dont la première montait rapidement et faisait songer à un "spike". Cette onde diphasique était suivie d'une autre, également diphasique, mais beaucoup plus lente. Sur la partie descendante de cette deuxième onde était parfois encore superposée une troisième onde plus petite (fig. 4.).

Ce phénomène que nous avons pu observer chez quelques Lamproies, est produit probablement aussi par la lésion du cœur, provoquée par la séparation du ventricule. D'ailleurs, Von Skramlik a déjà signalé à quel point les cœurs des poissons sont sensibles à toutes sortes de manipulations. Cette apparition d'une série de potentiels d'action diphasiques de l'oreillette est peut-être comparable au phénomène des "ge-

häufte Extrasystolen" décrit par VON SKRAMLIK, quand les systoles de l'oreillette se succèdent rapidement.

Comparé au ventricule, l'oreillette de la Lamproie est relativement grande et allongée; on y peut distinguer deux lobes: un lobe antérieur et un lobe postérieur. La forme compliquée de la courbe de l'activité électrique de l'oreillette isolée s'explique peut-être par le fait que les deux parties de cet organe ne battent pas simultanément. La forme compliquée de la deuxième et la troisième ondes semble indiquer dans ce sens.

Que ces courbes ne sont pas le résultat d'un éventuel artefact provoqué par un déplacement des électrodes fixées sur l'oreillette on a pu s'en

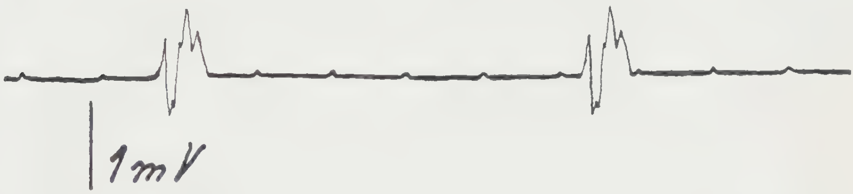

Fig. 4. Electrogramme de l'oreillette après la séparation du ventricule.

convaincre en répétant les mêmes expériences avec des électrodes ayant la forme de fins fils d'argent élastiques qui étaient fixées dans la paroi de l'oreillette.

Dans une de nos expériences le ventricule d'une Lamproie s'est spontanément arrêté, tandis que l'oreillette et le sinus continuaient à se contracter. En déterminant les potentiels d'action, les deux électrodes étant fixées sur l'oreillette on a enregistré une courbe dans laquelle une rapide onde diphasique était succédée par une autre, également diphasique, mais plus lente (fig. 5.). Comme le ventricule restait arrêté jusqu'à la fin de l'expérience (environ une heure), tandis que l'oreillette continuait à battre, nous supposons que les deux ondes sont d'origine auriculaires. Les potentiels d'action du sinus, très petits dans tous les cas, ne se voyaient pas sur cette courbe.

En dérivant l'activité électrique de l'oreillette isolée, nous avons fréquemment enregistré deux ondes diphasiques dans les électrogrammes. Le même cas se présentait dans les électrogrammes du ventricule.

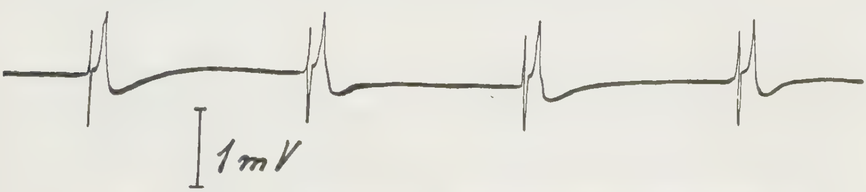

Fig. 5. Electrogramme de l'oreillette, enregistré après l'arrêt du ventricule.

Straub (1910) a été le premier à obtenir également des courbes à deux ondes en enregistrant l'activité électrique des oreillettes isolées du cœur de la Grenouille. Frédéricq (1912) a décrit le même phénomène pour l'oreillette droite isolée du Chien. Il signale que les phénomènes électriques de l'oreillette correspondent à ceux du ventricule et tout comme dans les courbes du ventricule, on peut distinguer dans celles de l'oreillette un "R" et un "T". Dans des circonstances normales, le "T" de l'atrium serait absorbé par le "QRS". Ce fait a de nouveau été confirmé en 1956 par les expériences faites par Rijbak et Retail sur le cœur de la Grenouille.

Les courbes à deux ondes obtenues dans les électrogrammes des oreillettes isolées, généralement d'un caractère diphasique, ont été décrites par Jolly (1916) pour la Tortue, Spadolini (1919) pour les Salamandres, et Rylant (1925) pour les Chats et les Lapins.

VI. CONCLUSIONS

Les expériences que nous avons faites sur des Lamproies ont donné les résultats suivants:

1. Par des dérivations des altérations du potentiel électrique, directement du cœur ou du péricarde isolé, on obtient des ECG qui présentent une grande ressemblance avec les ECG des Poissons et des Grenouilles. Dans la plupart des cas, on distingue les ondes P, QRS et T.

2. On obtient des ECG normaux du cœur des Lamproies lorsque le cœur est très soigneusement isolé. A la moindre lésion se manifestent des modifications plus ou moins prononcées dans l'activité électrique du cœur. Mais même en l'absence de lésions, l'ECG du cœur des Lamproies est très variable et dépend aussi de la position des électrodes.

3. La dérivation de l'ECG à partir de la peau ou des muscles donne de courbes dont les ondes sont extrêmement petites ou manquent totalement.

4. Des déterminations des électrolytes du sang de Lamproie ont démontré que la teneur en Na, K et Ca correspond approximativement à la teneur de ces ions dans la solution Ringer de la Grenouille.

5. Des déterminations des résistances électriques de la peau, des muscles et du péricarde cartilagineux ont démontré qu'elles ne se distinguent pas beaucoup des résistances de la peau et des muscles chez les Grenouilles.

6. Des dérivations des potentiels électriques dans les cas oú les deux électrodes étaient fixées soit sur l'oreillette, soit sur le ventricule, on a pu constater que la conductibilité des variations de potentiel électrique d'une partie du cœur à l'autre est relativement minime.

7. En enregistrant les potentiels électriques d'un cœur dont le ven-

tricule a été enlevé ou s'était arrêté, on obtient des électrogrammes où l'on peut distinguer deux ondes diphasiques; la première onde montre une évolution rapide et raide, la seconde une évolution plus lente.

Compte tenu des données de la littérature, nous sommes enclins à considérer ces ondes comme l'"R" et le "T" de l'oreillette.

8. Les expériences mentionnées ci-dessus indiquent clairement que le champ électrique du cœur de la Lamproie doit être très faible.

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THE SIGNIFICANCE OF THE BREAK-DOWN OF OAK LITTER BY ENOICYLA PUSILLA BURM.[1]

by

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The leaves falling in a forest every year during autumn build up a litter layer that is gradually attacked by a rich variety of organisms, ranging from bacteria of several μ to earthworms of more than 10 cm. This biological attack together with the effect of abiotic, physical and chemical influences form the decomposition process, which ultimately gives free minerals and CO_2—which are respectively leached or given off into the atmosphere—as well as humus substances which are very resistant to further decomposition.

In this decomposition process the biological attack is the most important, and because of the large variety of organisms involved, a very complicated one. To gain some insight into the mechanism of this process, it is necessary to evaluate the role of the most important groups of organisms, their succession and their mutual influence. In this paper the part played by a population of the terrestrial caddis fly larva *Enoicyla pusilla* Burm. (fig. 1) in the decomposition of the litter of oak coppice in 1957 will be analysed and also the influence of its activity on the subsequent decay of the litter material.

The adults of *Enoicyla* develop during September and October. Only the males have wings but they move only over short distances. They live for about two weeks, the unwinged females for about five days. Shortly after copulation the females deposit about fifty eggs in a gelatinous cover. The eggs hatch after about three weeks. The young larva immediately begins with the construction of its case (4).

On the first day the case already measured about 1.5 mm. In January the average length had increased to 3.3 mm, in March to 4.5 mm. In June the larva was full-grown and the case had a length

[1] It was intended to publish this paper in the supplement devoted to Prof. Dr. C. J. VAN DER KLAAUW. Unfortunately however, it was not possible to send in the manuscript in time.

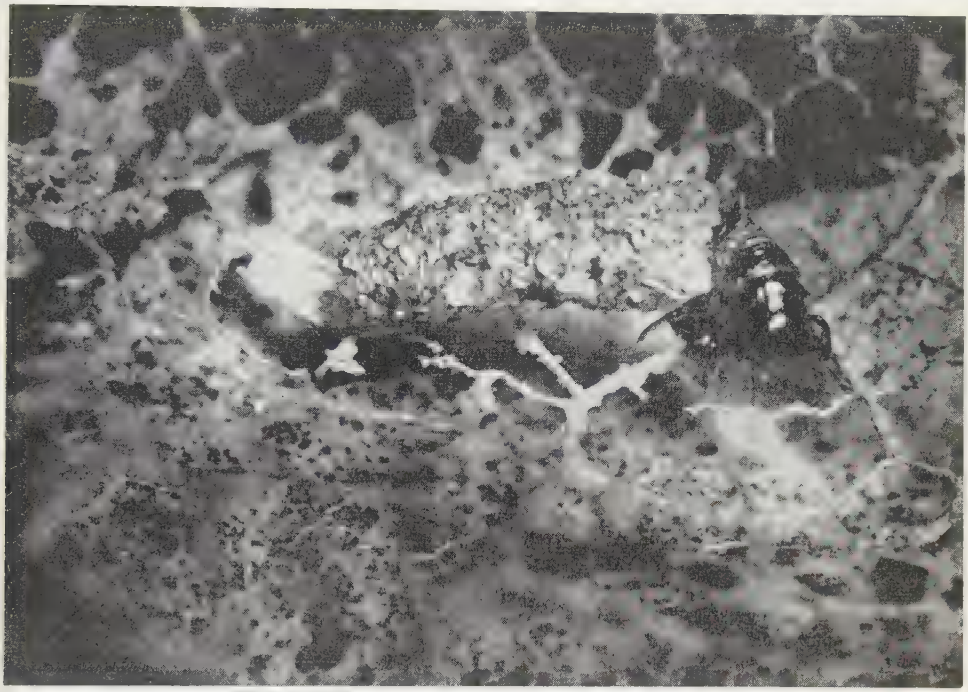

Fig. 1. Larva of *Enoicyla pusilla* Burm. on an oak leaf.

of 6.7 mm on the average. The dry weight of the larva increased from 0.1 mg in January and 0.25 mg in March to 1.3 mg in June. Most growth occurred during March, April and May. Till the end of June the larva moved around but then a silken wall was constructed across the posterior aperture of the case and the larva burrowed into the soil.

During August and September the larva pupated, after having closed the anterior aperture of the case as well. About a month later the pupae opened the anterior lid and crawled to the surface, where the imago emerged.

The larva lives during its feeding-time in the litter layer. Under sufficiently damp conditions it crawls around and feeds on litter, moss and algae. During the main feeding period, March, April and May the largest amounts of oak litter were found to be taken in. Numerous gaps from the underside of the oak leaves to the upper epidermis and a great many skeletonized leaves (fig. 1) showed the effect of the feeding activity of *Enoicyla*. To value the significance of this species for the break-down of the litter we have to know the following items:

1. The average litter consumption of the larva.
2. The number of animals in the field.
3. The litter production in the field.

From the weights of the larva as mentioned above, and the appearance of holes in the oak leaves it is obvious that the attack on the leaves is mainly restricted to a period of 100 days from March till June. So the considerations below are confined to this period.

In about the middle of this period a feeding experiment in petri-dishes with 5 batches of 100 animals on oak litter (moisture content 70%) showed that the average litter consumption per larva is 0.6 mg of air-dry litter per day. The average retention percentage (amount of food assimilated in percents of the amount of food consumed) is about seven, showing a very inefficient use of the food ingested.

It is interesting to compare the amount of assimilated food from the beginning of March till June with the growth during the same period. The litter consumption during this period approximates a hundred times the average daily litter consumption. Assimilation can be calculated to be $0.07 \times 100 \times 0.6 = 4.2$ mg on air-dry basis, that is 3.8 mg oven-dry, whereas the average growth during the period of 100 days is $1.35 - 0.25 = 1.1$ mg. The resulting growth/assimilation ratio of 29% seems to be very low in comparison with several values mentioned by LINDEMAN(2) e.g. in *Tubifex*, where this ratio was 62%.

First it must be remembered that during the hundred days the *Enoicyla* larvae moult several times and so the organic matter produced is more than the growth figure obtained by subtracting the weight at the start from the final weight. Indeed in short-term feeding experiments in April a weight increase of 1.65 mg per hundred larvae per day was found. In the second place no caloric values are used as in the case of *Tubifex*. The larger fat content of the animal substance, as compared with the feeding material, should raise the caloric value of the animal tissue formed during growth as compared with that of the assimilated food. In addition, terrestrial habits, case bearing and small feeding efficiency may be responsible for a high energy loss.

The amount of litter, produced during autumn was measured in seven catching nets of 0.5 m^2 each in the area of high density (800–1200/m^2) and in the area of lower density of *Enoicyla* (200–400/m^2). In the first area the average litter production per square meter was 325 g, in the second area 350 g. Of the total leaf fall 19% was consumed by the larvae in the first area and 4.5% in the latter. Taking into account the difference in litter production, these amounts agree proportionally with the densities in these areas. In the total area of occurrence of *Enoicyla*, 9% of the leaf fall was consumed by this species only.

A summary of the field data is given in table 1.

As a result of the chewing action of the larvae an average oak leaf is

broken down into about three thousand faecal pellets (0.8 × 0.45 mm) containing about ten million particles (56 μ diameter). This results in a twofold increase in volume and an exposed surface, about fifteen times as large as that of the original leaf. This means better aeration and increased water holding capacity. Furthermore it was established under natural conditions, that evaporation from the pellets is almost three times as low as from whole leaves.

TABLE I

Densities in different areas of the field	Surfaces of these areas in m²	Total number of larvae in thousands		Litter production in kg per m²	Litter production in kg total	Litter consumption of the population from March–June kg	Litter consumption of the population from March–June % litter prod.
200	2800	560	1080	0.35	1435	64.8	4.5
400	1300	520					
800	1000	800	2120	0.325	683	127.2	19
1200	1100	1320					
Total	6200	3200			2118	192	9

The result of the digestive action of the larvae on the oak leaf material is less striking (fig. 2). Most pronounced is the reduction of the content of easily decomposable carbo-hydrates as determined by the anthrone method(3). As a result of the preference of the larvae for non-lignified tissue the lignin content as determined by the thioglycollic acid method (1), is lower in the faecal pellets than in the original

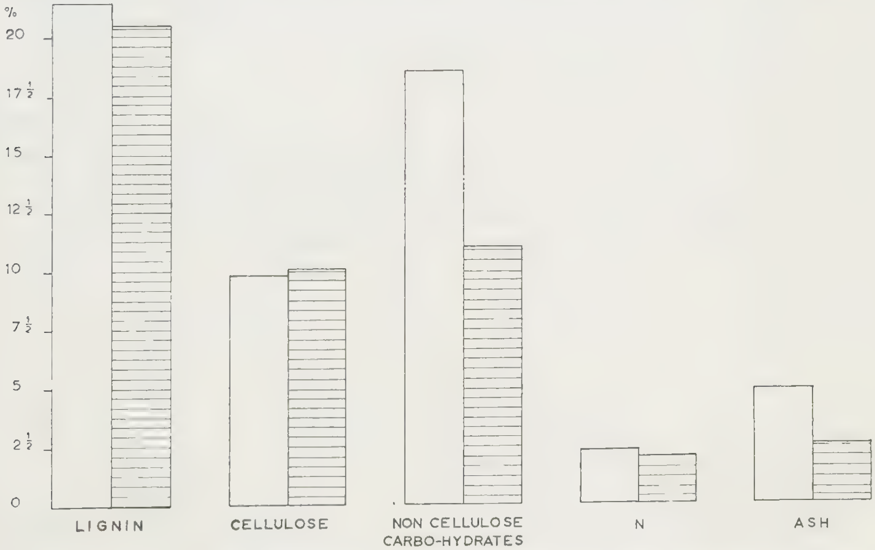

Fig. 2. Chemical constituents of oak leaves in % oven-dry (white columns) and faecal pellets (hatched columns).

leaves. The reduction in the ash content is probably due to the fact that the larvae exclude from their diet little grains of sand adhering to the leaves. The amount of cellulose, as determined by the anthrone method, after treatment with 75% H_2SO_4, is slightly higher in the pellets than in the food. This is probably also a result of the preference of the larvae for non-lignified tissue.

During passage through the intestinal track the pH of the material is raised from 4.3 as in the original leaves to 6.7 in the pellets. The physical changes in the leaf material, rupturing the cuticle and cell walls and exposing the cell contents, together with increased aeration and water holding capacity and decreased evaporation and a raised pH, render the faecal material a perfect substrate for microbial attack.

By suspending faecal material and mechanically produced particles of leaves of a similar size in suitable media (nutrient agar pH 6.8 and rose bengal-streptomycin agar pH 6.1 for bacteria and fungi respectively) almost 10,000 times as many bacterial and 50 times as many fungal colonies were obtained from the pellets than from the leaf particles.

Taking the evolution of carbon dioxide as an indication of microbial activity it was found that, during a period of 3 weeks at room temperature and under optimal moisture conditions, in fresh pellets the microbial activity is about seven times as high as in a comparable quantity of whole leaves (fig. 3). After a time-lag of about two days microbial activity in the pellets starts to increase and reaches a maximum about five days after excretion. After about three weeks the activity is

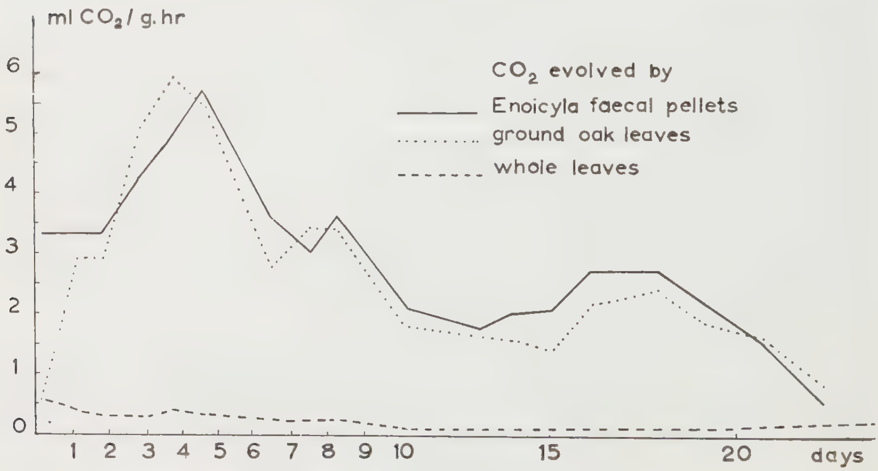

Fig. 3. Evolution of CO_2 from 1g oak leaf material in Conway units at 25° C (water was added after 1 and 2 weeks).

back to the level of that of the micro-organisms in the whole leaves. The importance of the mechanical break-down is underlined by the almost identical course of the evolution of CO_2 during the same period from leaf material, mechanically ground to the same particle size as in the pellets.

During this experiment the pellets did not show much mycelium development on their surfaces till after the maximum CO_2 production. From then on, mucoraceous fungi covered the pellets. After twelve days of incubation, bacterial counts were four times as high as in the fresh pellets but fungal colonies were over a hundred times as numerous (table II). Over the same period counts of bacteria in the ground leaves, had increased over a hundred times, and fungal colonies were over a thousand times as numerous. At the end of the three weeks of the experiment, fungi imperfecti were predominant on the pellets.

TABLE II

Number of colonies from 1 g of dry material (averages of duplicate series)

Material	Initial pH	Final pH	Incubation in days	Bacteria After 1 day	Bacteria Final count	Fungi After 1 day	Fungi Final count
Ground oak leaves	4.3	4.4	12	8.10^5	14.10^7	5.10^4	85.10^6
Faecal pellets of *Enoicyla pusilla*	6.7	4.4	12	7.10^9	30.10^9	26.10^5	33.10^7
Faecal pellets of *Glomeris marginata*	5.0	4.3	25	29.10^6	4.10^4	14.10^4	34.10^6

Unfortunately an accident prevented to investigate the chemical changes in the pellets caused by microbial activity. This was done in comparable material: the faecal pellets of the milliped *Glomeris marginata* Vill. fed on the same leaves as *Enoicyla*. In this material after three weeks of incubation at 25°C bacterial counts were very much reduced whereas fungal counts were over a hundred times higher. At the same time the pH of the pellets and leaf material had dropped to 4.3.

Comparing the chemical composition of the faecal pellets of *Glomeris*

TABLE III

Components of faecal pellets of *Glomeris marginata* incubated at 25°C. Percentage of oven-dry material

	Fresh pellets	After 6 weeks
Nitrogen	1.7	1.5
Cellulose	12.2	8.8
Non cellulose carbohydrates	10.1	11.2

before and after incubation in vitro for six weeks at 25°C, it was found that there was a sharp decline in the amount of cellulose whereas the percentage of easily decomposable carbohydrates slightly increased (table III). This is the normal picture in microbial decomposition of leaf material, since the loss of the easily decomposable carbohydrates from the substrate is compensated by carbohydrates formed in the microbial tissue. Contrary to the normal picture there was a decrease in nitrogen content, probably due to the loss of NH_3 during the early stages of decomposition of the neutral faecal material. The loss of cellulose over this six week period was about equal to the average loss of cellulose in the experimental oak wood plot, with an acid mull type during winter, spring and summer.

SUMMARY

The role of a population of larvae of the caddis fly *Enoicyla pusilla* Burm. in the decomposition of the litter in oak coppice wood in 1957 is described.

During the main feeding period the litter consumption varied, dependent on local density and litter production from 4.5 to 19% of this production. Only about 7% of the food consumed was assimilated. The remaining 93% are faecal pellets, which have a much larger exposed surface and a better aeration and water holding capacity than the original litter. The chemical changes are: less easily decomposable carbohydrates, a somewhat lower lignin and ash content and slightly higher cellulose content. Moreover the pH is raised from 4.3 in the leaves to 6.7 in the pellets. The microbial attack of pellets, original dead leaves and leaf particles of the same size as the particles in the pellets is compared by bacterial and fungal counts and CO_2 production. In the pellets bacteria were predonimant in the early stage and were replaced then by fungi. The effect of micro-organisms on faecal pellets after six weeks at 25°C, was a sharp decline in the amount of cellulose and a decrease in nitrogen content, whereas the easily decomposable carbohydrates remained almost constant.

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THE ROLE OF WATER CURRENTS IN THE ORIENTATION OF MARINE ANIMALS

by

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I. INTRODUCTION

In connection with the question whether marine animals can use hydrographical factors, such as salinity and temperature, for their orientation, the point is of interest whether they might be able to use for orientation the direction of water currents in case these displace water masses of favourable or unfavourable characteristics. It can easily be shown that horizontal gradients in salinity and temperature cannot as a rule be used for direct orientation, because the differences that should lead the animals into a certain direction are not sufficiently great to be perceived, while undisturbed gradients over large stretches of open sea are hardly to be found. It appears possible, however (VERWEY 1958), that an animal that remains at a certain place, and perceives changes in water characteristics there in the course of the tidal cycle, by facing the current or moving with it, is led to favourable areas. The animal would not make use of spatial hydrographical differences then, but of hydrographical differences in time. These would stimulate the animal to move with the current or against it. It would mean that the current so to speak would take over the function of temperature, salinity or other characteristics of the water as a means of orientation.

Should this principle exist, one would be inclined to suppose that it would be of general importance, and that it would also play a part with food seeking animals. An animal scenting a certain favourable substance could then find its food simply by facing the current, also in water in which a gradient in scent does not occur.

In order to test this supposition experiments were carried out with the nudibranchiate slugs *Aeolidia papillosa* and *Trinchesia aurantia*, which were offered the smell of their chief food in flowing water.

II. METHODS

The method used was a very simple one. The thing to be studied was the reaction of the animals to a current in flowing sea water containing the scent of their food, and in flowing sea water not containing this scent. The scent had to be present in a diffuse state and not as a gradient in order to exclude the possibility that the animals would use this gradient for orientation. It was supposed that the slugs would face the water current in case the water contained the scent and that they would not face it when no scent was there.

The first experiments were made in a long aquarium vessel through which a number of *Aeolidia papillosa*, placed on a glass plate hanging between bottom and surface of the vessel, were moved with their plate in the longitudinal direction of the aquarium. Such a movement, if sufficiently constant, should be felt by the slugs like a current in a direction opposite to that in which they were moved, notwithstanding the fact that the water did not move. It was supposed that the animals would remain in their places when they would be moved through ordinary sea water, and that they would move over their glass plate in its own direction of movement, i.e. against the current, when the water contained the scent of their food, present in a diffuse state.

The experiments in question were not successful, since the slugs reacted so slowly that a very long tank would have been needed to obtain good results. For that reason another method was tried.

On the bottom of a round glass aquarium of 30 cm diameter a round glass plate of 25 cm diameter, with a central opening of 10 cm diameter, was laid on three corks. The plate had been divided into 16 sectors by means of lines carved in it with a diamond, so that any displacement of the slugs could easily be ascertained. A circular water movement was generated by a propellor with two glass blades, driven by a small motor. The velocity of the water movement could be changed by means of a variable resistance and of different wheels between motor and propellor. To exclude the disturbing influence of light the experiments were carried out in the dark. The current velocities used were 2, 4, 8 and 12 cm per second over the outer part of the plate. The velocities were about half that value on the inner part and the average values may therefore have been about 1.5, 3, 6 and 9 cm per second.

Seawater containing the scent of the chief prey of the slugs was obtained by placing a number of these animals in an aquarium vessel for a certain period of time and by siphoning the water in question off. With this water the vessel containing the slugs was filled.

The slugs tested were *Aeolidia papillosa* and *Trinchesia aurantia*. The scent used was, in the case of *Aeolidia*, that of the anemone *Metridium*

senile, the favourite food species of this slug; in the case of *Trinchesia* the scent of *Tubularia indivisa* (later also that of *Tubularia larynx*) was used, the favourite food of *Trinchesia*. In each experiment 10 slugs were used, and since 10 experiments were made with *Aeolidia* at each current velocity, the total of slugs tested at each current velocity was 100; with *Trinchesia* the only current velocity used was an average velocity of about 6 cm/sec.

The above observations were made in 1953 by VAN HAAFTEN. The figures obtained by him refer to the numbers of slugs counted at the end of each experiment, after 20 minutes. Since all totals refer to 100 animals, the figures represent percentages at the same time.

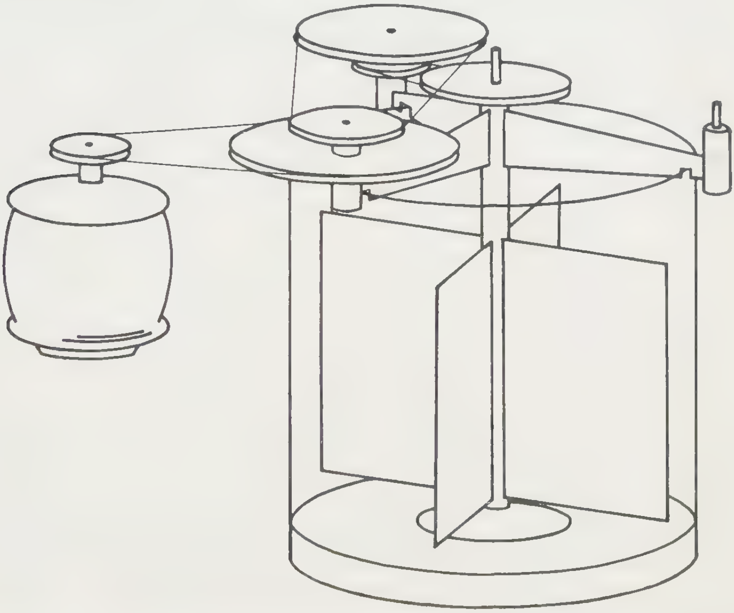

Fig. 1. Apparatus for producing different current velocities to test the influence of scent on the reaction of the slugs to currents.

In 1958 a permanent apparatus was made, so that the observations could easily be continued when opportunity offered (see Fig. 1). It did not virtually differ from the apparatus described, except that it is very easy to handle. To test it a few observations were made during the summer courses that year. They were made on *Trinchesia aurantia* and in this case water containing the scent in question dripped directly into the experimental vessel from another jar containing a small colony of *Tubularia*. The observations yielded similar results as those by VAN HAAFTEN. They are added to his.

III. OBSERVATIONS

The results of the observations on *Aeolidia* made by VAN HAAFTEN are summarized in table I.

The results of the observations on *Trinchesia aurantia* made by VAN HAAFTEN are summarized in table II.

The results of the observations on *Trinchesia aurantia* made in 1958 are summarized in table III. In this case only 27 animals were available,

TABLE I

Numbers (at the same time percentages) of *Aeolidia papillosa* moving against or with the current, or remaining in place, in water without or with the scent of *Metridium senile*

Average current velocity in cm/sec	Without or with scent	Number of slugs facing the current	Number of slugs moving with the current	Number of slugs remaining in place	Total number of slugs
1.5	without	30	51	19	100
	with	64	18	18	100
3	without	39	41	20	100
	with	95	1	4	100
6	without	45	26	29	100
	with	92	3	5	100
9	without	29	48	23	100
	with	54	4	42	100

TABLE II

Numbers (at the same time percentages) of *Trinchesia aurantia* moving against or with the current, or remaining in place, in water without or with scent of *Tubularia indivisa*. Average current velocity about 6 cm/sec

Without or with scent	Number of slugs facing the current	Number of slugs moving with current	Number of slugs remaining in place	Total number of slugs
without	57	33	10	100
with	85	4	11	100

TABLE III

Numbers (not percentages!) of *Trinchesia* moving against or with the current, or remaining in place (or crossing the current), in water without or with the scent of *Tubularia larynx*. Average current velocity about 2 cm/sec

Without or with scent	Number of slugs facing the current	Number of slugs moving with current	Number of slugs remaining in place	Total number of slugs
without	21	50	67	138 (6 exp. of 20–27 slugs each)
with	70	5	34	109 (5 exp. do)

their number decreasing to 20 in the course of the experiments; the observations were made in broad daylight and the scent used was that of *Tubularia larynx*. The periods of observation were very short, 5 minutes or less. The average current velocity was about 2 cm/sec.

IV. DISCUSSION AND SUMMARY

The results for *Aeolidia papillosa* show that in a weak current, without a scent indicating food, more slugs move with the current than against it. But when the current increases somewhat in strength more animals face the current than move with it. When the current is still stronger, the majority of the slugs move with it or stop moving.

When the water contains a scent that indicates food, while a gradient

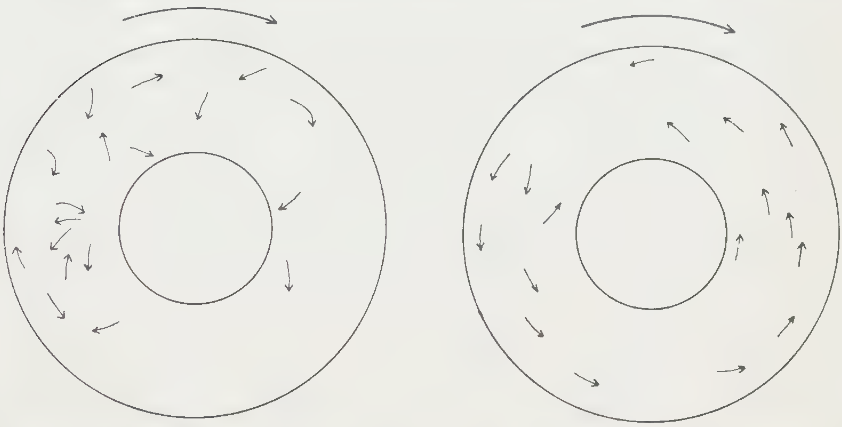

Fig. 2. Results of two experiments with *Trinchesia* in 1958. Left: without scent, right: with scent. The long arrows denote the current direction, the short arrows the direction of movement of the slugs. Current velocity about 2 cm/sec.

in scent is lacking, there is a distinct change in the behaviour of the slugs in that the majority now face the current (see fig. 2). This is especially the case at average current velocities of about 3 and 6 cm/sec. It is partly due to the fact that slugs that first stayed at their places, now join in, partly, however, to the fact that slugs that first move with the current now face it. The slugs therefore show a strong tendency to face the current as soon as there is food to be scented. When the average current velocity becomes about 9 cm/sec the percentage of slugs remaining at their places greatly increases; the animals apparently do not like to be on the move in water flowing with this velocity.

The results for *Trinchesia aurantia* obtained by VAN HAAFTEN,

referring to an average current velocity of about 6 cm/sec, do not differ from those for *Aeolidia papillosa*, except for the fact that the percentage of *Trinchesia* not on the move is the same both in water with and without *Tubularia*-scent. It is therefore still clearer than in the case of *Aeolidia* that the direction of movement in relation to the current direction changes with the presence of scent.

The results for *Trinchesia* obtained in 1958, referring to an average current velocity of about 2 cm, further corroborate the fact that the presence of scent makes the animals face the current. The reason why so many animals are not on the move is probably that the concentration in scent was much lower in these experiments than in those by VAN HAAFTEN. Moreover, *Tubularia indivisa* is preferred to *Tubularia larynx* (BRAAMS and GEELEN, 1953).

Before the experiments were started it was expected that the slugs in flowing water without the presence of scent indicating food would hardly move. They did move, however, and it has already been stated that the direction of their movement in relation to the current direction changed with the current velocity. This is especially shown in table IV.

TABLE IV

Percentage of animals moving *with* and *against* the current in scent-free water of different current velocities

Current velocity	*Aeolidia papillosa*		*Trinchesia aurantia*	
	With	Against	With	Against
1.5–2	51	30	36	15
3	41	39	—	—
6	26	45	33	57
9	48	29	—	—

From table IV we may infer that in weak currents both species of slugs studied show a tendency to move rather with the current than against it. When the current becomes a little stronger the majority of the animals face the current, but still stronger currents cause them to move with the current again. The results for *Trinchesia* are less complete than those for *Aeolidia*, but what results there are point in the same direction.

It is obvious that in strong currents weak animals like nudibranchiate slugs will prefer to move with the current and not to face it. But why do the animals prefer to move with the current when it is very weak and to face it when it becomes somewhat stronger? Tables I, II and III show us that the same principle holds good when the water does contain scent.

We think the general tendency for the slugs is to stay where they are

or to move with the current when it is very weak. Therefore, facing the current may be due to its stimulatory effect when the velocity increases and also to the fact that more scent particles are displaced per unit of time. In this connection, attention should be drawn to the fact that the water used in the experiments (also the so called scent free water) came from the seawater piping of the Station. Since the aquarium of the station contains numerous anemones, especially *Metridium senile*, the possibility should be left open that in the case of *Aeolidia* the positive reaction of the slugs in so called scent free water may have been due to the presence of small quantities of scent. Against this possibility may be advanced, however, that also in the case of *Trinchesia* in scent free water more animals faced the current than moved with it. In this case the water from the piping cannot have contained the scent of its prey, since the aquarium does not contain hydroids; it is known from the work of BRAAMS AND GEELEN (1953) that the scents of *Metridium* and *Tubularia* differ greatly, and that *Trinchesia* is not attracted by the scent of anemones. We therefore are inclined to believe that currents of a certain velocity by themselves already stimulate the animals to face them.

Our chief conclusion, however, is that it is especially the addition of food scent to flowing water, also if this scent is present in a diffuse state so that it cannot possibly be used as a gradient, that makes the slugs face the current. This means that the current takes over the role of orientating the animals, which in still water is carried out with the aid of a gradient in scent, so that the animals will automatically be led to the place of origin of the scent in question.

These results do not prove that the same mechanism is at work in the case of migratory animals using certain characteristics of the water for their orientation, but we now know that nature does use this principle, and it may well be of general value.

We are greatly indebted to Mr. A. D. G. DRAL, on whose instigation the permanent apparatus described above (fig. 1) was made.

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THE INFLUENCE OF TEMPERATURE ON GEOTAXIS AND PHOTOTAXIS IN LITTORINA OBTUSATA (L.)

by

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(Zoological Station, Den Helder)

I. INTRODUCTION

The species of the genus *Littorina* belong to the very best examples of a group of marine animals showing vertical zonation within the tidal area. One of the means by which they succeed in keeping to this area is that they possess a negative geotaxis, which holds them near the water surface. That we are dealing with geotaxis here has been shown for *Littorina neritoides* by Fraenkel (1927), wo found that they react on a centrifugal force in the horizontal plane by moving to the axis of the centrifuge. For several other species it has been demonstrated that a vertical movement upward is not only carried out in the light, but also in the dark, as well as in diffuse light, in which *photo*taxis does not play a role. That a negative geotaxis is important in these animals cannot, therefore, be doubted. Ebbinge Wubben and Barkman (Barkman 1955) made it seem probable that in the blunt periwinkle, *Littorina obtusata*, the negative geotaxis is counteracted by a strong preference for Fucaceae, on which this species feeds, and apparently also by a negative phototaxis in strong light.

Although data on negative geotaxis are so numerous, convincing data on positive geotaxis in *Littorina* are scarce. One might suppose that the strong negative geotaxis may at least undergo a change under the influence of temperature, since it is difficult to believe that a species like *Littorina obtusata*, inhabiting shallow water even in the far North, would not descend to somewhat deeper levels during severe cold. For that reason it seemed worth while to study temperature influence on geotaxis in this species. Although the study of temperature influence on *photo*taxis was not included in the original plan of the investigations, it will be seen below that it was automatically made part of the work soon after its start.

The investigations were carried out at the Zoological Station, Den Helder, from September to December 1955. I am greatly indebted to Mr. A. D. G. Dral, biological analyst of the Station, for his valuable help in working out the data, and to the director, Dr. J. Verwey, for suggesting the subject to me, and for his help in the redaction and translation of the paper.

II. METHOD

The animals used in the experiments were collected along the southern part of the island of Texel on 9, 24 and 30 November. Since BARKMAN was of opinion that the specimens of the yellow variety, *citrina*, differ in their somewhat weaker geotaxis from those of the green variety, *olivacea*, animals of only one colour, viz. yellow ones, were used. BAKKER (1959) since then made it seem probable that *citrina* and *olivacea* should not be considered separate varieties, as BARKMAN believed, but that they represent age stages, since the great majority of the young animals are yellow, while the older animals are either yellow or green.

The collected snails were kept in flowing sea water on *Fucus*. During the introductory experiments it became clear that they are very sensitive to putrefaction of the algae. As soon as the algae show signs of putrefaction the snails no longer attach themselves to them. For that reason the algae were removed every three days. As touching the snails has an unfavourable influence on them, this was avoided as much as possible.

The method of experimenting with the snails was a very simple one. They were given the opportunity to crawl on a glass plate of 16 × 52 cm, placed vertically in a rectangular aquarium vessel of 18×8×45 cm

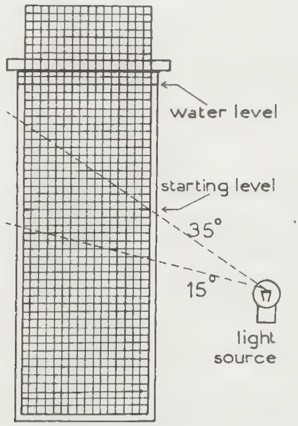

Fig. 1. Schematic representation of experimental upset. For explanation see text.

(fig. 1). The plate was higher than the aquarium, so that part of it (10 cm) emerged.

After the snails had been kept for some hours in water of the same temperature as was to be used in the experiments, the glass plate was taken from the vessel and laid down horizontally. After that, the snails (some 12 in each experiment) were placed on the middle part of the wet plate and allowed about 5 minutes to attach themselves. Part of them fell off the plate when it was placed vertically. These were not used that same day. The plate was hanging in the vessel by means of a glass strip glued on to its upper part. In this way it made nowhere direct contact with the aquarium walls.

In order to provide sufficient oxygen a slow current of sea water from the laboratory piping was kept running through the vessel. The water entered the vessel near the bottom, behind the vertical plate, while it overflowed at the surface, or, later on, was siphoned away, so that the water surface was kept below the upper edge of the vessel. The temperature was kept constant by heating the water electrically or cooling it by passing it through cooled calcium chloride.

Experiments were carried out at 15°, between 10° and 9°, at 5°, and at a number of temperatures between + 3.5 and — 1.2°C. It was impossible to work with temperatures below — 1.2°C. To make sure that the reaction of the animals did not change as a result of the season, experiments at 15° were also carried out simultaneously with the experiments at temperatures of 10°, 5° and lower. Thus, many more snails were tested at 15° than at the other temperatures. Temperature variation at 15°, 10° and 5° was 0.1°C; at lower temperatures it was maximally 0.5°, except in one experiment, in which the temperature fell from 3.5°C to 2°C.

On the surface of the vertical glass plate a network of square centimeters had been drawn so that it was easy to copy the course of the snails on millimeter paper. The course was noted down every 5 minutes for 60–70 minutes. The snails were marked by figures so that they could be individually identified; to this end Chinese ink was used after the shells had sufficiently dried up.

To prevent an arbitrary influence of light the experiments were carried out in a room from which daylight was excluded. Since Van Dongen had found, however, that the snails are little active in total darkness they were given a light of constant intensity. If under such conditions there is diffuse illumination the snails will move strictly vertically, but in the experiments a 60 watt bulb was placed beside the aquarium vessel in the plane of the vertical glass plate, at 15 cm from the vessel and 15 cm above the bottom. This caused the majority of the snails, which were now influenced both by gravity and by light, to move

obliquely. It will be seen that this gave the advantage that temperature influence on geotaxis and on phototaxis could be studied at the same time.

III. OBSERVATIONS

1. TREATMENT OF DATA

Since it was necessary to have a large number of observations to be able to express the results in general figures, and since the result of each observation was the pathway of one snail, numerous separate pathways, some 500 in all, were obtained. There is no sense in publishing all the

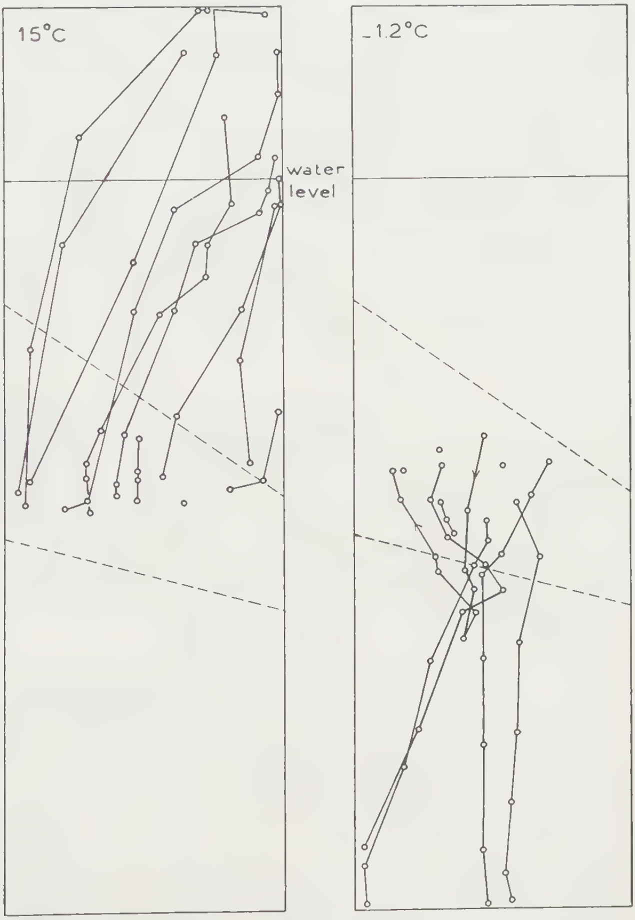

Fig. 2. Pathways of snails in two separate experiments. For explanation see text. Circles denote positions of the snails at five minute intervals.

observational material. Only the results of two separate experiments are given by way of illustration, while, further, a summary of the combined results is given in the form of a diagram.

The pathways for two groups of snails, observed in water of + 15° and — 1.2°C, respectively, are given in fig. 2. Of 11 snails, placed on the middle of the glass plate in water of 15°, 10 move obliquely upward in the direction of the lighted side, while one remains at its place. Most of the animals pass the water surface and continue their way up over the part of the plate that is emerged. Further, out of 9 snails tested in water of — 1.2°C, 5 move obliquely downward, one creeps obliquely downward and then obliquely upward, while 3 remain at their place. The movement of the snails is now mostly away from the lighted side, but part of the time it is more or less toward the light source.

It was shown in preliminary experiments that in diffuse light the general direction in which the snails move is vertically upward. It follows from the observations mentioned above that, when the light is not diffuse, the majority of the snails move upward with a deviation towards the lighted side. By means of a number of experiments, in which the light was placed to the left instead of to the right, it could easily be demonstrated that the light was indeed the cause of the deviation. It is therefore clear that the oblique pathways are the result of the combination of a negative geotaxis and a positive phototaxis. The direction thus tells something about the way in which geotaxis and phototaxis partake in the movement.

The snails placed on the plate received the light from a direction making an angle of about 25° with the horizontal. This angle changed of course considerably as the snails moved on. At the top of the plate it was something like 60°, at the base something like — 40°. In order to be able to denote the direction of the movement of the snails in relation to that of gravity and light only that part of the pathways of the snails was considered in detail which was lying in the area delimited by light directions making angles of 15° and 35° with the horizontal. This area has been denoted by two dotted lines in fig. 2.

Fig. 3 gives the combined results of all experiments in a diagrammatic form. It has been obtained in the following way from the pathway-observations. Of each 5 minute-part of each pathway falling with its greater part within the area delimited by the 15° and 35° lines the direction was noted with reference to the horizontal, 90° representing vertically down, 270° representing vertically up, etc. Each value obtained is represented by a dot in fig. 3, in which the temperatures of the experiments are given on the horizontal axis, the direction of movement on the vertical axis. Animals that had crept along the vertical edge of the plate were not counted, the same applies to snails that had

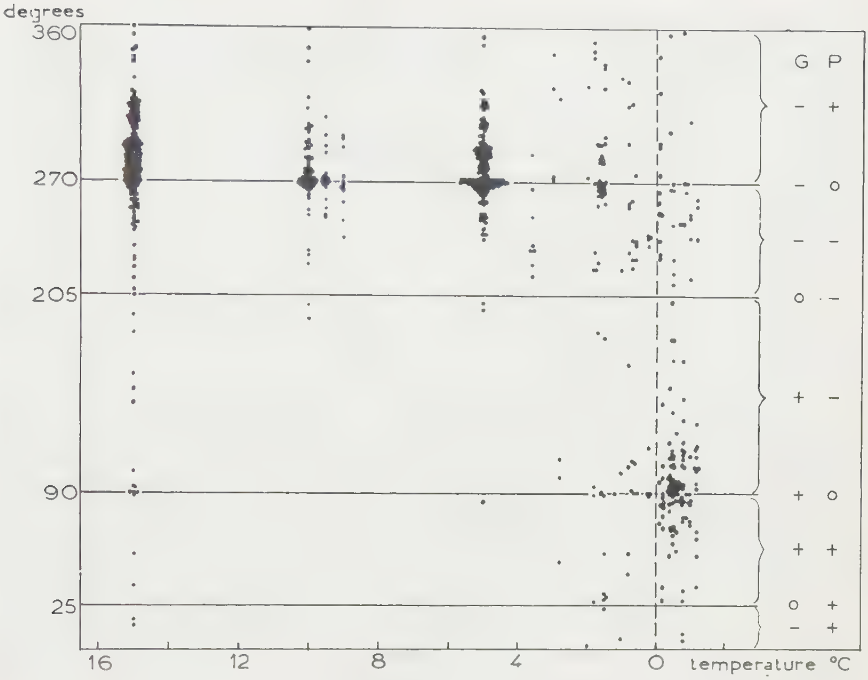

Fig. 3. Diagrammatic representation of results. For explanation see text.

met each other en route. Snails which had not moved and had remained at their place were not counted either.

Fig. 4 may serve to explain the signs of geotaxis and phototaxis for the different angles dealt with in fig. 3. If a snail moves straight up or downward, the light has no effect on the direction of movement at all. If it would move straight towards the light or away from it (25°, resp. 205°), gravity has no influence on the direction of movement. Therefore,

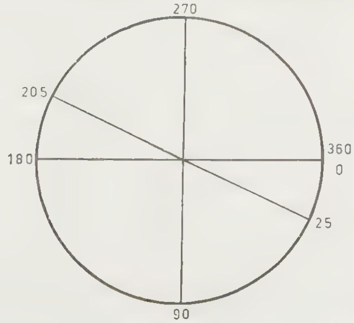

Fig. 4. Diagram explaining the sign of geotaxis and phototaxis as denoted in fig. 3

in the directions between 270 and 25° geotaxis is negative and phototaxis is positive; in the directions between 270° and 205° geotaxis and phototaxis are both negative; in the directions between 205° and 90° geotaxis is positive, whereas phototaxis is negative; in the directions between 90° and 25° both geotaxis and phototaxis are positive. These notations are mentioned at the right side of fig. 3.

2. GEOTAXIS AND PHOTOTAXIS

It is at once clear from fig. 3 that in water of 15°, 10° and 5°C the majority of the snails move straight up, with a deviation to the lighted side, which may amount to more than 40°. Part of the snails however (those between 270 and 205°) deviate away from the light. This may mean that the reaction to light varies much among different animals. A small number of the snails move down instead of moving up, but such animals are only found in water of 15°; one would like to know if the higher temperature has something to do with this fact.

Between 3.5° and zero the percentage of upward moving animals deviating to the left increases. Apparently, attraction by the light diminishes somewhat. From 3° downward there is apparently also an increase in animals moving down instead of moving up; in other words, the sign of geotaxis begins to show a change there.

Below zero the proportion of positively geotactic animals greatly increases. Still, part of the animals travel up. Lower temperatures than — 1.2°C could not be obtained, but it is probable that around — 1.7°C (the freezing point of sea water) all animals will show positive geotaxis. The animals that still move up in water of below zero are mostly negatively phototactic, and there can therefore be no doubt that the sign of phototaxis changes at about the same temperature as the sign of geotaxis. Among the animals moving downward there is a small majority showing negative phototaxis; many of them, however, move towards 90°, thus showing that they are apparently little influenced by light.

Adding all results together, negative geotaxis drives the animals up at all temperatures above about 3°, but the observations at 15° may indicate that geotaxis becomes positive above that temperature. From about 3°C downward the number of positively geotactic animals gradually increases, until at — 1.2°C most animals show positive geotaxis. The majority of the negatively geotactic animals react positively on the light source used in these investigations, whereas the majority of the positively geotactic animals is either weakly influenced by light or negatively phototactic. This apparently means that at low temperatures

positive phototaxis is replaced by negative and neutral reactions and that the lower the temperature the more important geotaxis relatively becomes.

The existence of a true geotaxis in marine animals has more than once been doubted. KANDA (1916) supposed that an upward movement must be due to the weight of the shell, which would direct the animal vertically. FRAENKEL already refuted this possibility by making the shell lighter by means of wax: the upward movement then continued. Here, KANDA's supposition is refuted in another way, since at low temperature all animals move downward head-first.

3. THE VELOCITY OF MOVING

The velocity of movement of the snails is higher in water than in air, and it is higher at the higher temperatures. Fig. 5 has been obtained by averaging the 5 minute-distances for different temperatures. At 15° the average velocity in water is over 17 mm/min., below zero it is about 7 mm. One would expect that the velocity becomes very low towards zero,

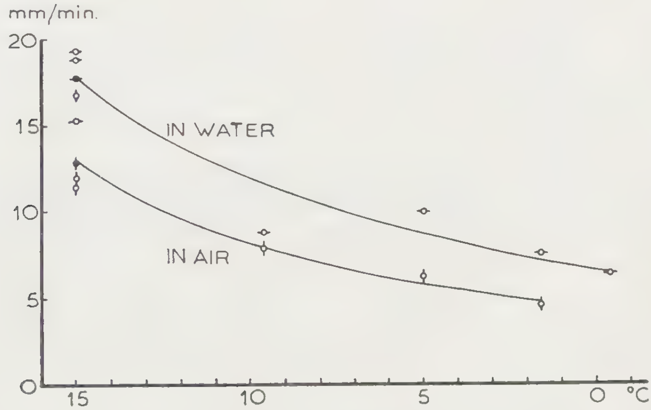

Fig. 5. Velocities of movement of snails at different temperatures in water and in air. Open circles denote averages of observations, black circles denote averages of the separate results for the 3 series studied at 15°C. In calculating the last averages the numbers of animals that contributed to each of the separate values were taken into account.

but the average velocity hardly falls below 7 mm. This is of much importance in connection with the fact that a movement away from the surface starts only near zero.

The average velocities show a clear difference in water and in air, those in air being lower. The under-water weight must be lower due to

the buoyancy, while also the friction under water will be lower. Both these factors may be of influence.

4. THE INFLUENCE OF THE WATER SURFACE

Although the sign of the geotactic movement does not change until the temperature approaches zero, the influence of temperature fall becomes apparent much sooner in the aversion of the snails to cross the water surface and to travel a certain distance through air. This is especially evident from table 1, in which the number of snails stopping at the boundary between water and air is expressed as a percentage of all animals which reach the water surface or cross it.

TABLE I

Influence of water surface

Temperature in °C	Stopping at water surface in %	Crossing water surface in %	Total number of animals
15	4	96	133
10	4	96	39
5	17	83	53
0–5	44	56	25
below 0	100	0	5

It is clear that the water surface, which is easily crossed at 15° and 10°, especially forms a barrier at the lower temperatures. Also the distance travelled through air decreases at the lower temperatures.

IV. DISCUSSION AND SUMMARY

When starting, our chief aim was to discover whether the upward movement, shown by *Littorina obtusata* and other *Littorina* species, may reverse under the influence of a change in temperature. Such a reversal would be quite natural, since the sign of, e.g. phototaxis, is known to be influenced by temperature, and why would not the same apply to geotaxis? The observations showed that reversal indeed takes place.

What is most striking, however, is the relative suddenness of the change from negative into positive geotaxis. We had expected that this change would make itself evident very gradually, but the contrary is true. There are no indications that negative geotaxis at temperatures approaching zero would become less and less pronounced, and that the positive movement taking its place would develop gradually. One gets the impression that the number of snails moving up as well as down, or

showing a weak positive or negative geotaxis, shows no increase. It is certain that the number of snails that remain in their place does not increase at low temperatures. There is a sudden change in the numbers of animals moving down instead of moving up. Between $+1°$ and $-0.1°C$ half of 22 animals move down, the other half still move up, whereas between $-0.1°$ and $-1.2°C$ more than 90% of the 54 animals move down. It is therefore clear that the increase in positive geotaxis below $+2°C$ is due to the fact that around zero more snails abruptly start moving down instead of moving up. Ecologically, it is of much interest that the sudden change in the sign of geotaxis takes place around zero, since sea water with a salinity of about 30—35‰, as inhabited by this species, freezes at -1.5 to $-1.7°$. Through the effect of this mechanism the animals are kept in the upper water layers till the very last moment; they do not leave their safe *Fucus*-zone before the danger of freezing of the water is close by.

The velocity of movement of the animals decreases considerably (about 50%) when the temperature falls from 15°C to zero. It is quite striking, however, that at zero it still is about 7 mm/min., a fact of much interest in connection with the necessary displacements around that temperature.

Although the sign of the geotactic movement does not change until the temperature approaches zero, the influence of temperature fall makes itself apparent much sooner in the aversion of the snails to cross the water surface and to travel through air. One might ask whether this may indicate a decrease in the strength of the urge to move upward.

The observations at 15°C give the impression that geotaxis may also become positive above 15°. It would be of importance to study the reactions described in this paper at somewhat higher temperatures.

It is of interest that the sign of phototaxis changes at about the same temperature as the sign of geotaxis. Under the light conditions used in these investigations phototaxis is positive from about 3° upward. Compared with the influence of gravity, however, the influence of light is relatively small and at the lower temperatures its influence decreases still more, so that below zero there is only a small majority of negatively phototactic animals, most of the snails being symmetrically divided around 90°.

The influence of light has not been studied at other intensities than that used in our experiments. Such a study could give us more insight into the relative influence of light and gravity at different temperatures. It would be comparable to the study of CROZIER AND WOLF (1928), who used the change in the direction of movement of *Agriolimax*, as a result of the decrease in phototactic sensitivity with constant geotactic sensitivity, to get insight into light adaptation in this species.

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POPULATION STATISTICS OF FIVE SPECIES OF THE BAT GENUS MYOTIS AND ONE OF THE GENUS RHINOLOPHUS, HIBERNATING IN THE CAVES OF S. LIMBURG

by

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CONTENTS

I. INTRODUCTION

In 1956 we published a paper (SLUITER, VAN HEERDT and BEZEM, 1956) on the population statistics of *Myotis mystacinus*. In the present paper a similar study is made on four other species of the same genus: *M. emarginatus*, *M. dasycneme*, *M. daubentonii* and *M. myotis*, and on *Rhinolophus hipposideros*, which were banded and recaptured in the same cave area during about the same period as *M. mystacinus*. For particulars on the purport of this investigation and for general technical and mathematical considerations we refer to our publication on *M. mystacinus*, mentioned above.

In chapter II of the present paper additional data on periods of investigation, sampling caves and sample sizes are given, followed by supplementary mathematical considerations (chapter III). In the chapter on results (IV) special attention is paid to points of difference with our previous paper. In chapter V, results are recapitulated and discussed. Finally (chapter VI), life tables are derived from some of the estimates.

II. PERIODS OF INVESTIGATION, SAMPLING CAVES AND SAMPLE SIZES

In a comparative study on the population statistics of several bat species, hibernating in the same cave area, it is obviously desirable that the data refer to the same period of investigation and the same sampling caves for all species. On the other hand, sufficiently large numbers and a reasonably constant sampling activity are necessary conditions, that may interfere with a strict application of this rule. Actually, we had to compromise on several points, allowing slight differences in the choice of sampling period and sampling caves for different species.

These differences are caused mainly by irregularities in the sampling during the earlier part of the investigation. Although BELS (1952) started bat banding in the cave area already in 1938/39, it was not before 1940/41 that he came to visit regularly the places we have called sampling caves (*cf.* SLUITER, VAN HEERDT AND BEZEM 1956, p. 65).

Moreover, in that year and the next only the species *M. myotis* and *M. dasycneme* were banded, while the other species to be discussed in this paper were not included until 1942/43.

A further complication was introduced by the fact that Slavante, our most important sampling cave, was not sampled regularly before 1945/46. Consequently, for each species we had to choose between two possibilities: starting at one of the earlier dates mentioned above and excluding the data from Slavante, or starting at 1945/46 and including the data from Slavante. In each case we have chosen the alternative yielding the largest initial numbers. This turned out to be the first alternative in the case of *M. myotis*, *M. dasycneme* and *Rh. hipposideros*, and the second alternative in the case of *M. mystacinus*, *M. emarginatus* and *M. daubentonii*.

In the year 1944/45 banding activities were restricted by war circumstances. We thought it advisable to omit the incomplete data of this year. The year 1947/48 falls out too, because in this year no banding has been done.

The end of each period is defined by the stopping of our banding activities. This decision was made if, considering either the amount of information accumulated or the possible damage to the population caused by our banding activities, we felt continuation of the investigation no longer justified. This point was reached at different dates for different species.

The reader will observe, that the treatment of the data of *M. mystacinus* in the present paper differs from that in our previous paper. One year (1956/57) has been added, while on the other hand the first three years have disappeared. We consider the present treatment as more rigorous. Moreover, the calculation of the sex ratio has been corrected for regional differences in the rate of capture (see chapter III, par. 4). The differences in the estimates of the parameters of the population, however, are only slight, with the exception of the juvenile mortality (see chapter IV, par. 4).

For an enumeration of the sampling caves we refer to our previous paper (p. 65). As it is impossible to study all these caves separately, since the number of bats captured is generally too small, we were obliged to combine the data of the various caves. Only the cave Slavante contains a sufficiently large number of bats to justify a separate treatment of the data. Moreover, this cave has some peculiar properties from an ecological point of view to make this separation interesting. Therefore, we will distinguish in the following two regions: Slavante and the other caves combined.

Table I shows for each of the 6 species the period of investigation, the number of samples upon which our estimates are based, and the total

sample size, to give an impression of the number of bats involved in the present investigation.

TABLE I

Periods of investigation, numbers of annual samples and total sample sizes

Species	Period of investigation	Number of samples	Slavante			Other caves		
			♂	♀	♂ + ♀	♂	♀	♂ + ♀
M. myst.	45/46–56/57	11	427	316	743	755	330	1085
M. emarg.	45/46–54/55	9	249	220	469	680	459	1139
M. daub.	45/46–56/57	11	245	241	486	199	235	434
M. myot.	40/41–54/55	13	—	—	—	354	296	650
M. das.	40/41–56/57	15	—	—	—	623	568	1191
Rh. hipp.	42/43–55/56	12	—	—	—	914	803	1717

III. MATHEMATICAL CONSIDERATIONS

1. INTRODUCTION

In our previous study of the population statistics of the bat *Myotis mystacinus* a mathematical model of the population was devised and methods were given for the estimation of certain parameters from recapture data. Populations of other species of bats were studied likewise, and turned out to differ on several important points from the population of *M. mystacinus*. The differences necessitated a slight generalization of the model and some alterations in the methods of estimation, which will be discussed in this chapter.

As far as possible, the same notation as in the previous paper will be used. A short survey of the symbols and their meaning may be given here:

$m(t)$, $f(t)$ = relative frequency of males and females of age-class t;

m, f = relative frequency of males and females of all ages;

$p_m(t)$, $p_f(t)$ = probability of survival during one year of males and females of age-class t

$\sqrt{p(0)}$ = probability of survival during the first half year of new-born animals;

q_m, q_f = probability of being caught of males and females.

2. PROPERTIES OF A POPULATION CHANGING IN SIZE ONLY

a. *Size of the population as a function of time*

In the case of *M. mystacinus* we had to deal with a population that could be considered as stationary. Statistical tests, based upon the numbers of males and females in the annual samples, showed no significant change in either the size of the population or the sex ratio during the period of

investigation. The recapture data were compatible with a constant probability of survival and, finally, it could be argued that the age-distribution too must have remained unchanged.

Most of these conditions have been found in other species of bats as well. Constancy of size, however, proved to be an exception: in the majority of cases the populations were decreasing rapidly. The formulae, used to calculate age-distribution and juvenile mortality, had to be adapted accordingly. They will be derived here under the assumption that age-distribution, sex ratio and probability of survival are constant throughout the period of investigation. The size of the population, however, may vary from year to year.

Let $N(x)$ be the size of the population at time x, and $N(x+1)$ the size at time $x+1$, the interval of time being one year. Consider the males of age-class t at time x. Their relative frequency is $m(t)$ and, consequently, their number is $m(t)N(x)$. The probability of survival being $p_m(t)$, the number of survivors of this class after one year is expected to be $m(t)p_m(t)N(x)$. But as these survivors constitute age-class $t+1$ of the population at time $x+1$, their number must be equal to $m(t+1)N(x+1)$. Thus we obtain the relation

$$m(t)p_m(t)N(x) = m(t+1)N(x+1) \tag{1}$$

for males, and in the same way

$$f(t)p_f(t)N(x) = f(t+1)N(x+1) \tag{2}$$

for females.

If the relations (1) and (2) are valid, that is if the underlying conditions mentioned above are realized, the population must increase or decrease in geometrical progression. This can easily be seen by noting that the ratio

$$\frac{N(x+1)}{N(x)} = \frac{m(t)p_m(t)}{m(t+1)} = \frac{f(t)p_f(t)}{f(t+1)}$$

is independent of the time x. Consequently, the size of the population increases or decreases each year with a constant factor. Denoting this factor by the symbol c, we can write relations (1) and (2) as

$$m(t)p_m(t) = c\,m(t+1) \tag{3}$$

and

$$f(t)p_f(t) = c\,f(t+1) \tag{4}$$

b. *Age-distribution and juvenile mortality*

As far as the evidence of the recapture data goes, the conclusion seems justified that in all species of bats that were observed the probability of survival of adults is independent of age and sex. Therefore, we will

restrict the discussion to the special case that $p_m(t) = p_f(t) = p$ for all values of $t \geqslant 1$, in which case relations (3) and (4) are simplified to

$$m(t+1) = m(t)\ \frac{p}{c} \tag{5}$$

and

$$f(t+1) = f(t)\ \frac{p}{c} \tag{6}$$

Formulae for the calculation of the age-distribution can be derived from (5) and (6) in the same way as in the case of a stationary population. For particulars the reader is referred to the paper on *M. mystacinus*, p. 70–71. In the present case the following results are obtained:

$$m(t) = m(1-\frac{p}{c})\ (\frac{p}{c})^{t-1} \tag{7}$$

$$f(t) = f(1-\frac{p}{c})\ (\frac{p}{c})^{t-1} \tag{8}$$

The calculation of the juvenile mortality, too, remains essentially the same as in the case of a stationary population (see the paper on *M. mystacinus*, p. 71–73). The only important difference is, that in the case of an increasing or decreasing population we have to consider numbers instead of relative frequencies, since the latter refer to populations of varying size.

Consider the new-born animals produced by females of the population at time x. Their number will be $\sum_t n(t)f(t)\sqrt{p}\,N(x)$, males and females taken together. The survivors after the first half year constitute age-class 1 of the population at time x + 1. Their number will be

$$\{m(1) + f(1)\}\,N(x+1) = (m+f)\ (1-\frac{p}{c})\ c\,N(x) = (c-p)\ N(x).$$

Dividing the latter number by the former, we find for the probability of survival during the first half year:

$$\sqrt{p(o)} = \frac{c-p}{\sum_t n(t)f(t)\sqrt{p}} \tag{9}$$

Assuming $n(t) = 1$ for all values of $t > 1$, and $n(1) = 0$ (cf ch. IV, par. 4), the value of $\sqrt{p(o)}$ is given by:

$$\sqrt{p(o)} = \frac{(c-p)\,c}{f\,p\,\sqrt{p}} \tag{10}$$

3. ESTIMATION OF THE PROBABILITY OF SURVIVAL AND THE RATE OF CAPTURE

No essential change has occurred in our method of estimating the probability of survival and the rate of capture from the recapture data. It is still based (*cf.* the paper on *M. mystacinus*, p. 74–75) upon the fitting of straight regression lines to the points obtained by plotting the logarithm of the fraction of recapture against time. It will be necessary, however, to discuss in more detail the question of differences between the sexes or between different regions of the sampling area.

In general, the data from which the probability of survival and the rate of capture have to be estimated are given in a table of the following kind:

x	y	z
1	y_1	z_1
.	.	.
.	.	.
.	.	.
n	y_n	z_n

where y and z are logarithms of fractions of recapture after x years, referring either to males and females in the same region, or to animals of the same sex in different regions.

It is essential for the method to be described, that the number of points in the two series is the same. In many cases, however, in one of the series there are more points available than in the other, owing to the larger number of captured animals. In those cases, points that cannot be matched with a point in the other series are omitted.

The first question to be answered is, whether the 2n points can be represented by a single regression line or by two regression lines differing significantly either in slope or in level. This point can be decided by means of an analysis of variance the details of which need not be elaborated here. If a single regression line suffices, the probability of survival as well as the rate of capture has the same value in both series. Otherwise, there are differences in one of the two parameters or in both.

In the second place there is the question of estimation. Though in any case the estimates are based on the method of maximum likelihood, the formulae differ according to the outcome of the foregoing test. If there are no significant differences either in the probability of survival or in the rate of capture, the estimates are given by

$$\log p = \frac{n(\Sigma xy + \Sigma xz) - \Sigma x(\Sigma y + \Sigma z)}{2n\Sigma x^2 - 2(\Sigma x)^2} \tag{11}$$

$$\log q = \frac{\Sigma y + \Sigma z - 2\Sigma x \log p}{2n} \tag{12}$$

If there is a significant difference in the rate of capture only, so that we must distinguish two values q_y and q_z, the estimate of p is still given by (11), but (12) is replaced by

$$\log q_y = \frac{\Sigma y - \Sigma x \log p}{n} \tag{13}$$

and

$$\log q_z = \frac{\Sigma z - \Sigma x \log p}{n} \tag{14}$$

The third case, a significant difference in the probability of survival, need not be considered here, since it did not occur in any of the species of bats observed.

4. ESTIMATION OF THE SEX RATIO

Constancy of the sex ratio has been maintained as one of the assumptions underlying the generalized model of the population set forth in the second paragraph. The validity of this assumption has to be verified before an estimate of the sex ratio can be made. In our investigation of the population of *M. mystacinus* we used for this purpose the χ^2-test, but this test has the disadvantage that it is rather too exacting. We wish to test constancy of the sex ratio against a systematic trend only, and the χ^2-test leads already to rejection of the hypothesis if there are rather large fluctuations, but no systematic trend. For this reason the χ^2-test has been replaced by the more appropriate rank correlation test of KENDALL.

As regards the estimation of the sex ratio we will discuss here the corrections that are necessary if there are differences in the rate of capture. Obviously, if the two sexes have different rates of capture, the sex ratio cannot be estimated directly from the numbers of males and females in the samples, but each number has to be weighted with a factor $1/q$, where q is the rate of capture in question. The same correction must be applied if there are different rates of capture in different regions. In the most general case, let the rate of capture of males and females be $q_{m,1}$ and $q_{f,1}$ in region 1, and $q_{m,2}$ and $q_{f,2}$ in region 2. Then, if the numbers of males and females captured are a_1 and b_1 in region 1, and a_2 and b_2 in region 2, the estimate of the fraction males in the population is given by

$$m = \frac{a_1/q_{m,1} + a_2/q_{m,2}}{a_1/q_{m,1} + a_2/q_{m,2} + b_1/q_{f,1} + b_2/q_{f,2}} \tag{15}$$

5. ESTIMATION OF THE FACTOR OF INCREASE OR DECREASE

If the fact, that the size of the population increases or decreases during the period of investigation, has been established by means of KENDALL'S

rankcorrelation test, it remains to obtain an estimate of the rate of change. Naturally, the method of estimation depends upon the law according to which the size of the population changes. The present model of the population postulates a geometrical progression. Unfortunately, the fluctuations in the sample size are too large to allow a verification of this assumption with the exclusion of other possibilities. We have to take the fact for granted as long as no evidence to the contrary presents itself.

For the estimation of the factor c a distribution-free method has been chosen. It is carried out as follows. First the sample sizes are corrected for differences in the rate of capture in the way described above. Then the logarithms of the corrected sample sizes are plotted against time, yielding n points that should be represented by a straight line, the slope of which is equal to log c. For each of the $\frac{1}{2}n(n-1)$ pairs of points the slope is calculated and, finally, the median of this set of values is taken as an estimate of the slope of the line and, consequently, of log c.

IV. RESULTS

1. SURVIVAL RATES AND RATES OF CAPTURE

a. *Fraction of recapture as a function of time*

In the figs. 1–6 the fraction of recapture is plotted logarithmically against the number of years after banding. As pointed out on p. 517, straight regression lines are fitted to these points and, by means of the formulae (11)–(14), estimates of the survival rate and of the rate of capture can be obtained from the slope of these lines and from their intersection with the vertical axis, respectively.

To increase the accuracy of these estimates we have excluded those points for which the expected number of recaptures falls short of a certain minimum value. This minimum number depends upon the number of regression lines by which the points are represented. In those cases, where no significant differences between the sexes or between different regions are found and, consequently, all points are fitted satisfactorily by a single regression line, we have fixed, more or less arbitrarily, the minimum number at 10. In those cases where, owing to sex differences or regional differences, 2 or even 3 regression lines are needed, the same accuracy is obtained if the minimum number is fixed at $10/\sqrt{2} = 7$ or $10/\sqrt{3} = 6$ respectively.

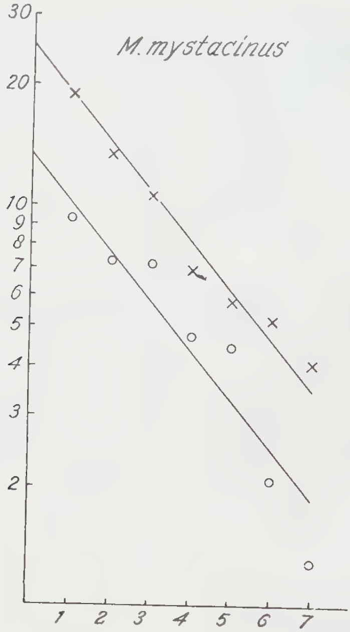

Fig. 1

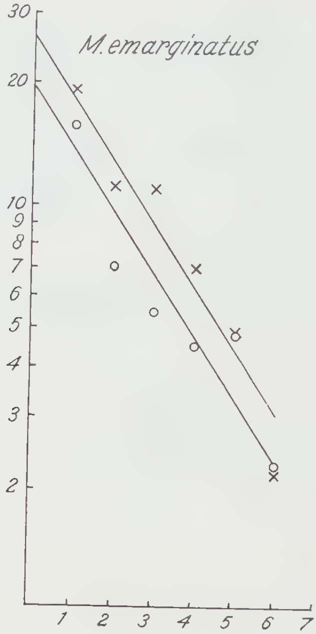

Fig. 2

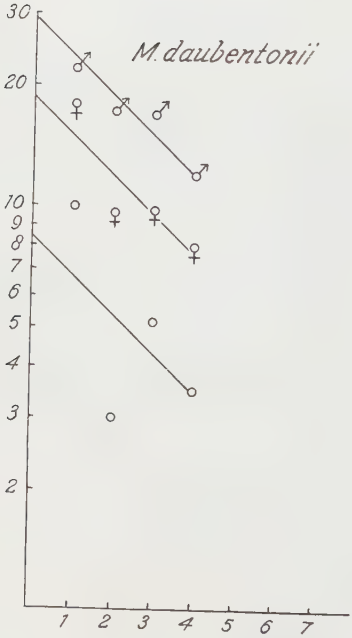

Fig. 3

Figs. 1–3. Fraction of recapture multiplied by 100 plotted logarithmically against the number of years after ringing in Slavante (x) and in the other caves combined (o). In *M. daubentonii* (fig. 3) males and females in Slavante are plotted separately; in the other cases the sexes are combined.

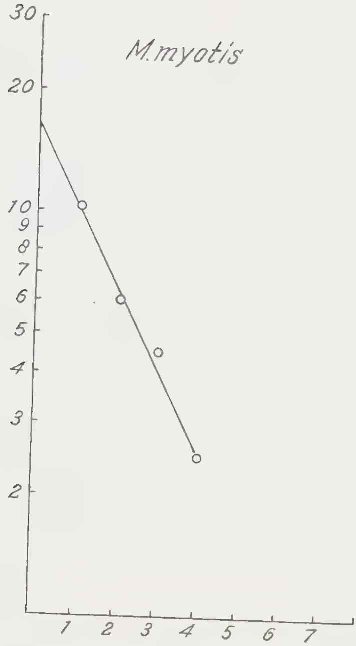

Fig.4

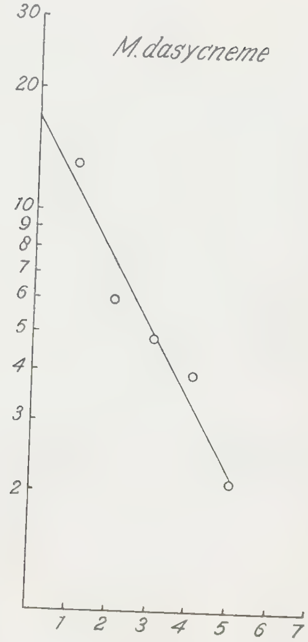

Fig.5

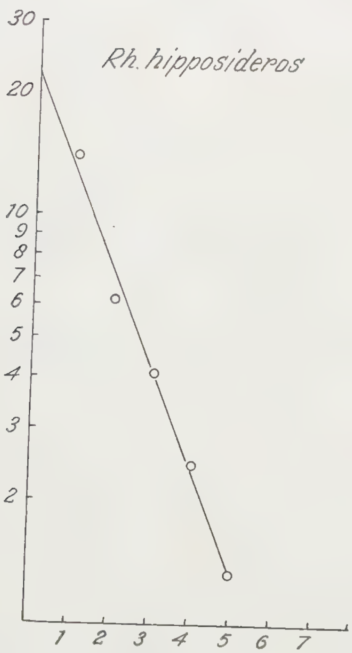

Fig.6

Figs. 4–6. Fraction of recapture multiplied by 100 plotted logarithmically against the number of years after ringing in the sampling caves, Slavante excluded; sexes combined.

b. *Sex differences*

Before estimates of the survival rate and the rate of capture can be made, it has to be established whether there are differences between the sexes or between different regions.

Sex differences have been tested first, in those cases, where different regions could be distinguished, in each region separately. Starting with two regression lines, one for males and one for females, an analysis of variance shows, whether these lines differ significantly in slope or in level. The analysis results in a value P for both properties separately indicating a significant difference between the sexes if it is $\leqslant 0.05$. The values found are recorded in table II.

TABLE II

Values of P for sex differences in slope and level of the regression lines

Species	Slavante		Other caves	
	Slope	Level	Slope	Level
M. myst.	0.10–0.20	0.50–0.70	0.20–0.30	0.10–0.20
M. emarg.	0.80–0.90	0.10–0.20	0.30–0.50	0.10–0.20
M. daub.	0.30–0.50	< *0.01*	0.90	0.80–0.90
M. myot.	—	—	0.80–0.90	0.70–0.80
M. das.	—	—	0.50–0.70	0.10–0.20
Rh. hipp.	—	—	0.10–0.20	0.30–0.50

From table II the following conclusions may be drawn.

1. In no case there is a significant difference between the sexes as regards the slope of the regression lines. Consequently, in all cases males and females have the same probability of survival.

2. Only *M. daubentonii* in Slavante shows a significant difference in level; in all other cases the sexes do not differ significantly in this respect. Owing to the fact that there are no differences in slope, this conclusion may be transferred directly to the intersection of the regression lines with the vertical axis. Consequently, males and females may be said to have the same rate of capture, except in the case of *M. daubentonii* in Slavante.

We do not want to lay too much stress on the difference found in the last-mentioned case. Naturally, we have to accept the outcome of the statistical analysis and we will be obliged, therefore, to base our subsequent calculations on different regression lines for males and females of *M. daubentonii* in Slavante. The argument in favour of a real difference is, however, considerably weakened by the fact that the same species does not show the phenomenon in the caves outside Slavante. After all,

there remains a chance, though it is smaller than 0.01, that the phenomenon in Slavante is due to sampling effects.

c. *Regional differences*

Where possible, regional differences between Slavante and the other caves combined have been tested in the same way as sex differences. In all cases where no sex differences were found, the recapture data of males and females have been combined to increase the initial numbers and, consequently, the number of points to each regression line. In this way, for the species *M. mystacinus* (fig. 1) and *M. emarginatus* (fig. 2) two regression lines were compared, one for the combined sexes in Slavante and one for the combined sexes in the other caves. For the species *M. daubentonii* (fig. 3) three regression lines were compared, one for males in Slavante, one for females in Slavante and one for the combined sexes in the other caves. The other three species (fig. 4–6) do not appear in this connection since no data from Slavante have been used (*cf.* chapter II). The results of the analysis are recorded in table III.

TABLE III

Values of P for regional differences in slope and level of the regression lines

Species	Slope	Level
M. myst.	0.20–0.30	< 0.001
M. emarg.	0.30–0.50	0.05–0.10
M. daub.	> 0.90	< 0.01

From table III the following conclusions may be drawn.

1. There are no significant regional differences as regards the slope of the regression lines. Consequently, in both regions the same survival rate holds.

2. In two cases the difference in level is significant, in the third case (*M. emarginatus*) it is on the verge of significance. For the same reason as before the same conclusion holds for the intersection of the regression lines with the vertical axis.

Consequently, the rate of capture in Slavante is different from that in the other caves, at all events in the case of *M. mystacinus* and *M. daubentonii*, and probably too in the case of *M. emarginatus*.

d. *Estimates of the survival rate and the rate of capture*

In accordance with the preceding results, the recapture data can be represented by a single regression line in the case of *M. myotis*, *M. dasy-*

cneme and *Rh. hipposideros*, by two parallel regression lines in the case of *M. mystacinus* and *M. emarginatus* and by three parallel regression lines in the case of *M. daubentonii*. In all cases, except that of *M. daubentonii*, the lines have been calculated with the data for males and females combined.

The regression lines, together with the points upon which they are based, are drawn in the figs. 1–6. In most cases the lines represent the points satisfactorily, in no case there is an indication that a curved line would fit better.

The simplest explanation of this fact is the assumption, that the survival rate and the rate of capture have remained constant throughout the period of investigation (*cf.* SLUITER, VAN HEERDT and BEZEM, 1956, p. 76).

Estimates of these quantities are recorded in table IV. For each species a single value of the survival rate, valid for both sexes and in both regions, is given. Of the rate of capture, however, one, two or three values are given as the case may need.

TABLE IV

Estimates of survival rate and rate of capture

Species	Survival rate	Rate of capture	
		Slavante	Other caves
M. myst.	0.752	0.251	0.133
M. emarg.	0.701	0.254	0.191
M. daub.	0.800	0.293 (m.) 0.186 (f.)	0.084
M. myot.	0.637	—	0.158
M. das.	0.667	—	0.168
Rh. hipp.	0.567	—	0.225

2. SEX RATIO

a. *Constancy*

In the figs. 7–12 the fraction males is plotted against the year of sampling. The individual points have been connected by a broken line for illustrative purposes only. Before an estimate of the sex ratio in each population as a whole can be made, it has to be established whether it is constant throughout the period of investigation and whether there are regional differences or not.

Constancy of the sex ratio has been tested by means of KENDALL's rank correlation test (*cf.* p. 518) in those cases, where different regions

could be distinguished, in each region separately as well as in both regions combined. Each test results in a value P, indicating a significant trend in the sex ratio if it is $\leqslant 0.05$. The values found are recorded in table v.

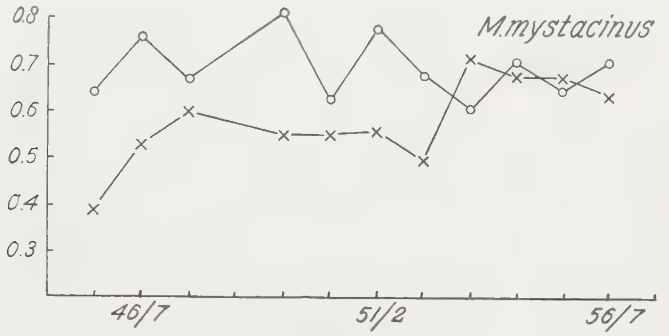

Fig.7

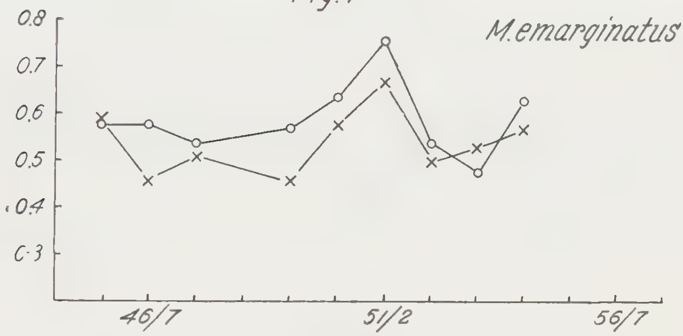

Fig.8

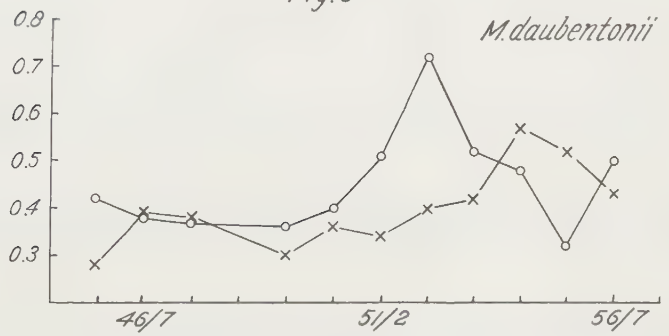

Fig.9

Figs. 7–9. Fraction males plotted against the year of sampling in Slavante (x) and in the other caves combined (o).

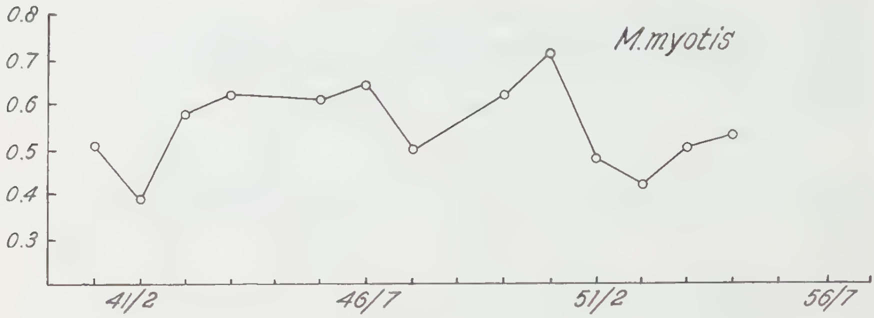

Fig.10

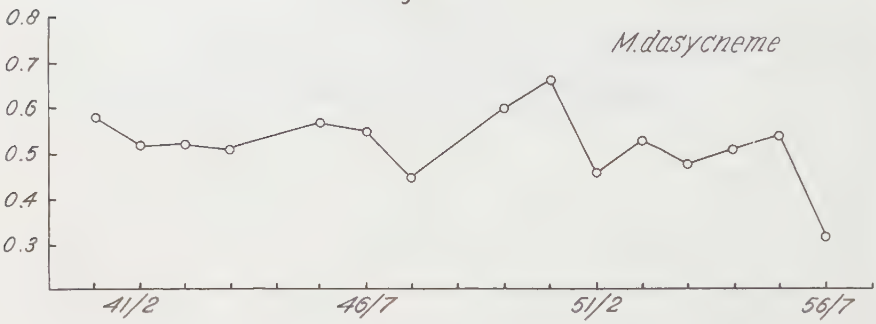

Fig.11

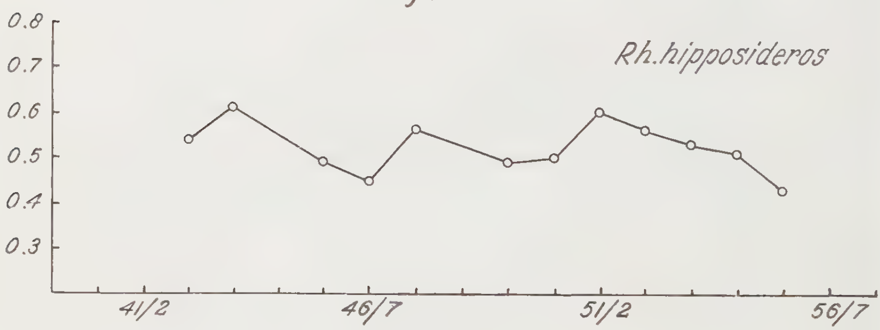

Fig.12

Figs. 10--12. Fraction males plotted against the year of sampling in the sampling caves, Slavante excluded.

Table v shows, that in the caves outside Slavante the sex ratio may be considered as constant in all species concerned. In Slavante, however, the species *M. mystacinus* and *M. daubentonii* show a significant trend, which from the figs. 7 and 9 may be specified as an increase in the fraction males.

TABLE V

Values of P resulting from test of constancy of sex ratio

Species	Slavante	Other caves	All caves
M. myst.	*0.026*	1.000	0.648
M. emarg.	0.761	0.919	0.761
M. daub.	*0.010*	0.542	0.060
M. myot.	—	1.000	—
M. das.	—	0.239	—
Rh. hipp.	—	0.459	—

It is difficult to explain this phenomenon since it does not occur in the caves outside Slavante, and there are no reasons to support the idea that we have to deal with two entirely separate populations. On the contrary, we prefer to think of each species as a single population, which only during the winter season splits up in a number of small groups choosing different hibernating caves.

For this reason, we have also applied the rank correlation test to the data from all caves combined, corrected in the way described above (p. 518) for differences in the rate of capture. The last column of table V shows, that from the combined data no systematic trend in the sex ratio can be proved, though it must be admitted that in the case of *M. daubentonii* the result is on the verge of significance. In our subsequent calculations we have considered the sex ratio as constant in each species and, consequently, used a single value characteristic for each population as a whole.

b. *Regional differences*

In the case of *M. mystacinus* and *M. emarginatus* the problem of testing the difference in sex ratio between Slavante and the other caves combined presents no difficulties. It is adequately solved by applying the normal approximation to the hypergeometric distribution. The results, recorded in table VI, prove that the sex ratio in Slavante differs significantly from that in the other caves.

In the case of *M. daubentonii* the test is complicated by the fact that a significant difference in the rate of capture of males and females in

TABLE VI

Values of P for regional differences in sex ratio

Species	P
M. myst.	$<$ *0.001*
M. emarg.	*0.016*

Slavante has been found. A test, dealing satisfactorily with this situation, does not exist. We have to be content with noting that the actual data, as shown in fig. 9 and expressed by the estimates mentioned below, suggest that in the species *M. daubentonii* the same phenomenon occurs as in the foregoing two, the fraction males in all these cases being lower in Slavante than in the other caves combined.

c. *Estimates*

Estimates of the fraction males in Slavante, in the other caves and in all caves combined have been computed by means of formula (15) (*cf.* p. 518). They are recorded in table VII. In subsequent calculations only the values of all caves combined will appear as population characteristics.

TABLE VII

Estimates of the fraction males

Species	Slavante	Other caves	All caves
M. myst.	0.575	0.696	0.664
M. emarg.	0.531	0.597	0.581
M. daub	0.392	0.459	0.439
M. myot.	—	0.545	—
M. das.	—	0.523	—
Rh. hipp.	—	0.532	—

3. POPULATION SIZE

As we prefer to think of each species as a single population (*cf.* p. 527), we did not investigate the changes in the population size in Slavante and the other caves separately. Both the tests of constancy and the calculation of the rate of change were carried out with the pooled data from all caves, naturally taking into account differences in the rate of capture. The denominator of the fraction in formula (15) (p. 518), applied to the yearly samples, was used as a measure of the population size. Though this quantity for statistical reasons cannot be considered as an unbiased estimate of the population size, its bias does not affect the results of the test nor the value of the rate of change. In the figs. 13–18 the values of the population size are plotted logarithmically against the year of sampling.

By means of KENDALL's rank correlation test it was established, whether the population size could be considered as constant or not. In the latter case, the rate of change was calculated according to the method described in chapter II (p. 519). The results of the test (P) and the values of the factor of yearly change (c) are shown in table VIII.

TABLE VIII

Values of P and c for change in population size

Species	P	c
M. myst.	0.648	1.000
M. emarg.	0.002	0.893
M. daub	0.218	1.000
M. myot.	< 0.001	0.925
M. das.	0.011	0.921
Rh. hipp.	< 0.001	0.887

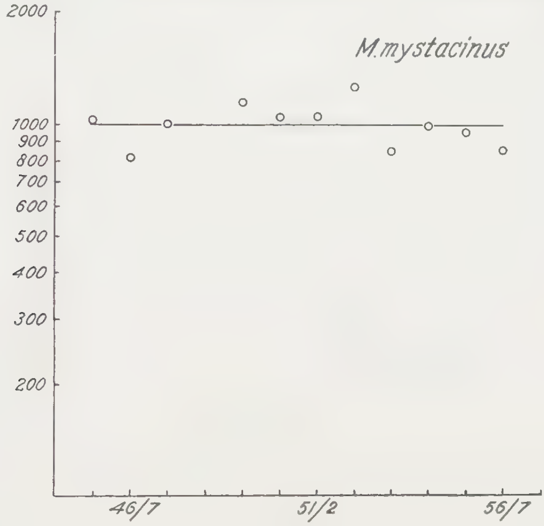

Fig. 13

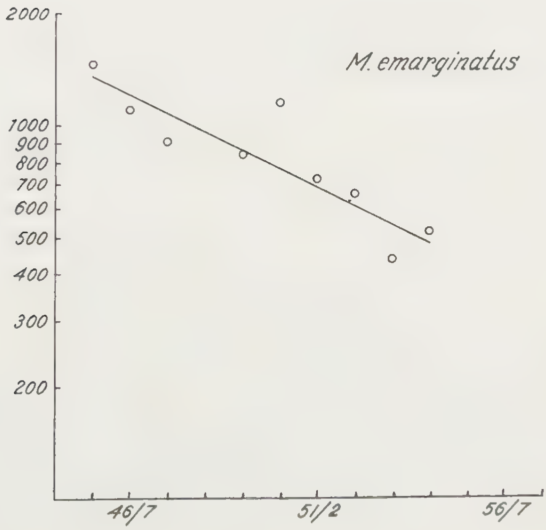

Fig. 14

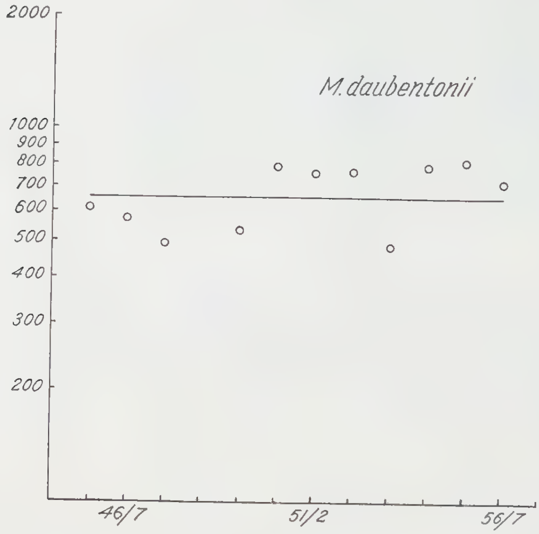

Fig. 15

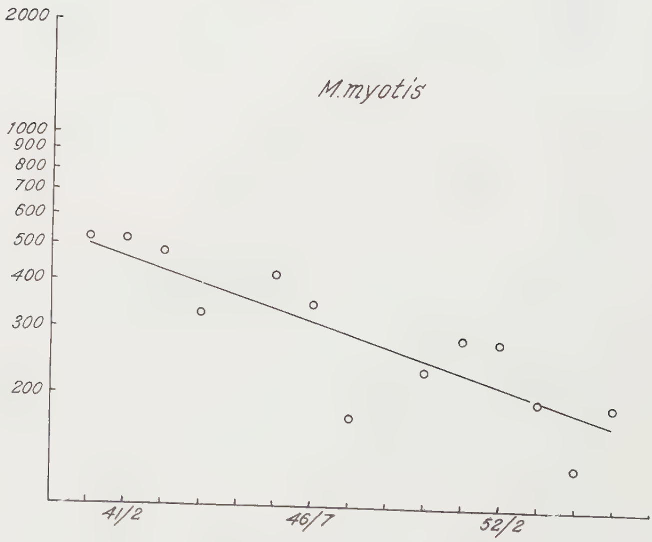

Fig. 16

Figs. 13—16. Population size in all caves combined, plotted against the year of sampling.

Only the populations of *M. mystacinus* and *M. daubentonii* appear to be constant in size, corresponding with a value of c = 1. The other four species show a significant decrease of approximately 10% annually. The corresponding regression lines have been drawn in the figs. 13–18.

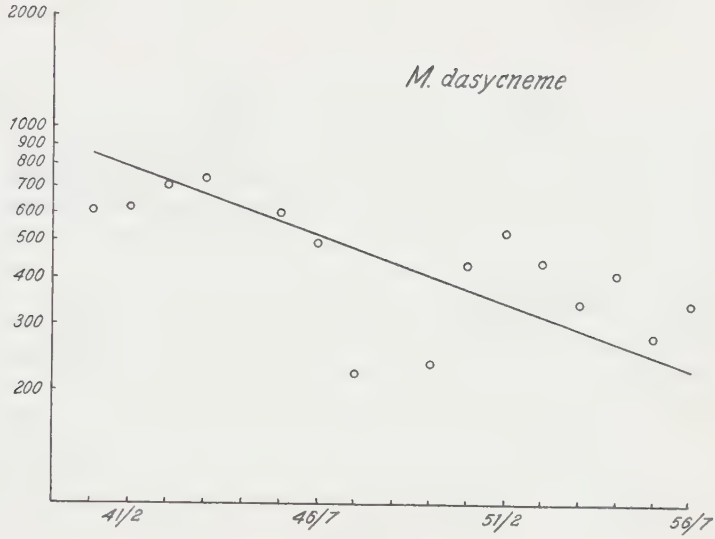

Fig. 17

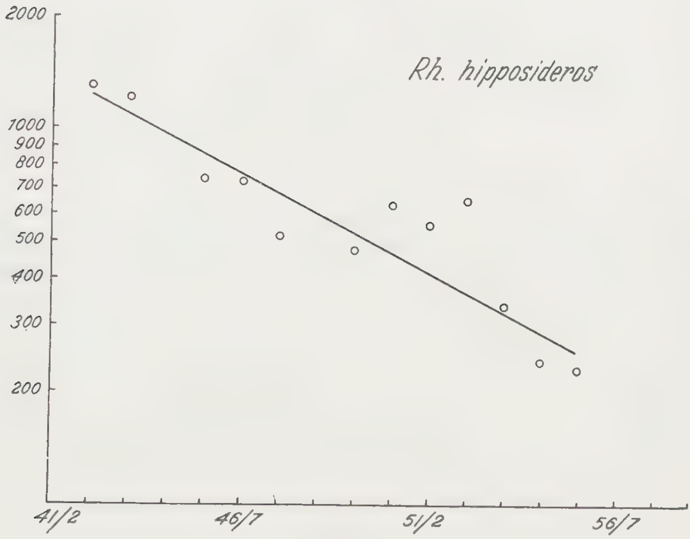

Fig. 18

Figs. 17 and 18. As in figs. 13—16.

4. JUVENILE MORTALITY

The calculation of the juvenile mortality in this paragraph will be based upon a reproduction rate of one young per annum for each reproducing female. A possible exception to this rule in the case of *Rh. hipposideros* will be discussed elsewhere. Therefore, only results in the genus *Myotis* are given below.

In our previous paper on *M. mystacinus* we calculated two extreme values of the juvenile probability of survival, the lower one corresponding to the hypothesis that females of all age-classes are reproducing, the upper one corresponding to the hypothesis that only females of age-class 2 and higher are reproducing. In the present paper we will consider only the latter possibility, since in the genus *Myotis* no information favouring the former could be obtained.

The juvenile probability of survival was calculated by means of formula (10) (p. 516). In two species an impossible result (> 1) was obtained. The results in the remaining species are recorded in table IX.

TABLE IX

Probability of survival during the first half year ($\sqrt{p(o)}$)

Species	$\sqrt{p(o)}$
M. emarg.	0.697
M. daub.	0.499
M. das.	0.900

V. DISCUSSION

In the preceding pages we have recorded a number of estimates of population properties (*cf.* tables IV, VII, VIII and IX) in 6 species of bats. When judging these results and comparing them with each other, we must be well aware of the fact, that their accuracy depends on several quantities, which have different values in different species. First, owing to differences in initial numbers between various species, the number of points on which regression lines could be based varies considerably and, secondly, in some species the dispersion of the points is much larger than in others (*cf.* figs. 1–6 and 13–18).

In addition, some other facts remain unknown or uncertain, which can possibly bias the results, recorded above. For instance, it is very likely that the sexes have different habits in choosing a quarter to hibernate, which will lead to a bias in our estimate of the sex ratio. Moreover, we must not forget that our results on surviving relate to banded individuals only. In generalizing these results over the entire population, it is assumed that no lethal damage is caused by banding (*i.e.* that no "ring mortality" occurs) an assumption, which cannot be verified easily in most cases.

In this chapter we will recapitulate and discuss our conclusions, and review the evidence available on these questions.

I. SURVIVAL RATE OF ADULTS

Before comparing results in different bat species, we will consider once more the accuracy of the methods used. In a previous paper (Sluiter, Van Heerdt and Bezem, 1956, table 3, p. 77) we have proved that our estimate of the survival rate has a standard deviation of 0.024–0.050, even under the rather idealized conditions of a sampling experiment with a population model consisting of punchcards. Estimates of the survival rate, originating from sampling data of real bat populations under natural conditions (as in the present paper) will be less accurate and, consequently, will have a standard deviation higher than 0.024–0.050.

Moreover, there is some evidence that two of our estimates of adult survival rate (table IV) are too low as a consequence of ring mortality, i.e. in the cases of *M. myotis* and *M. dasycneme*. We mentioned before (Sluiter and Van Heerdt, 1957) the fact, that serious injuries to the forearm may be involved in both species, when these rather big bats bite their ring. Our estimate of adult surviving of banded *M. myotis* is 0.637. Eisentraut (1950) calculated this rate as about 0.60, using recapture data from a population of the same species, individuals of which were banded with exactly the same type of rings as ours. We are inclined to believe that the estimate by this author is biased, for the same reason, by ring mortality.

In all cases we found the same rate of survival for males and females. No evidence turned up for the supposition of Eisentraut (1950), that females are more vulnerable than males, as a consequence of pregnancy and the nursing of their young.

Generally speaking, there is no doubt that the adult survival rate in bats is considerably higher than 0.50, and, therefore, higher than it is in most small mammals. This may be partly explained by two circumstances: first, that bats are not seriously suffering from predation, and, secondly, that they are able to evade unfavourable climatological influences by sleeping for long periods in each season.

In *Rh. hipposideros* the survival rate is lower than in the genus *Myotis*, as far as it is investigated here. This lower rate of survival is not correlated with a higher rate of decrease of the population size as one would expect. This can be explained by a higher reproduction rate in *Rh. hipposideros*, which will be discussed elsewhere.

Regarding the three *Myotis* species, in which no evidence of ring mortality exists, the survival rate is significantly lower in *M. emarginatus* than in *M. daubentonii* and in *M. mystacinus*, even when taking into account the standard deviation mentioned above. This is in accordance with the decrease in population size found in the former species, while the population of the latter two remained constant in size.

2. RATE OF CAPTURE

In the caves of S. Limburg the rate of capture is rather low (table IV), if compared with quarters, where bats hibernate in clusters (*cf.* EISENTRAUT, 1950 and TWENTE, 1955). This is due to habits as spreading over a wide subterranean area in S. Limburg and hiding in crevices. Moreover, we have the impression that the bats are changing readily from sampling caves to non-sampling caves, and vice versa, which lowers their rate of capture also (*cf.* SLUITER, VAN HEERDT and BEZEM, 1956, p. 66).

In every case but one we found the same rate of capture in males and females. The apparent difference in *M. daubentonii* in Slavante (table II) is supposed to be not real (*cf.* p. 522). We conclude, that males and females have the same habits in choosing a hiding place in the cave, where they entered in order to spend the winter. This does not exclude the possibility, which is discussed below, that other quarters—maybe other places than caves—are favoured especially by females during the hibernation period too, as is known to be the case during the summer.

3. SEX RATIO

In all cases, except in *M. daubentonii*, we found the sex ratio disproportionate in advantage to the males (table VII).

This seems to be a rule for bat populations, when hibernating in caves (*cf.* DAVIS, 1959). According to this author, the possibility that more males than females are born is hardly tenable. In addition, we have found no evidence that males have a higher rate of survival than females. So, in our case, there seems to remain only one factor which could be responsible for the greater number of males: many of the females spend the winter outside the sampling caves. It is not easy to verify this assumption, as the hibernating quarters, favoured especially by females may occur everywhere in the total distribution area of the population involved. DAVIS (1959) found that the sexes of the American bat *Pipistrellus subflavus* have different habits in choosing a quarter to hibernate. We found differences in sex ratio between Slavante and the other caves, indicating that the same phenomenon exists in the species concerned here.

4. REGIONAL DIFFERENCES

Only differences between Slavante and the other caves combined could be investigated and then in three species only (p. 513).

In both regions the same survival rate holds. This could be more or less expected as we have probably to deal with one population, the

individuals of which concentrate during the hibernation period in equivalent caves in a limited area of about 50 square miles.

The rate of capture is higher in Slavante than in the other caves. This may be ascribed to properties of the caves considered which are mentioned in a previous paper (SLUITER, VAN HEERDT and BEZEM, 1956).

The fraction males is higher in Slavante than in the other caves. In the same time the fraction males shows in two cases (*M. mystacinus* and *M. daubentonii*) a tendency to increase in Slavante. We can only signalize these phenomena here, as we are not able to explain them from the ecological point of view.

5. POPULATION SIZE

The populations of *M. emarginatus*, *M. myotis*, *M. dasycneme* and *Rh. hipposideros* show a decrease of approximately 10% annually. The dispersion of the points (*cf.* figs. 14, 16, 17 and 18) does not allow conclusions concerning possible differences in rate of decrease between different species.

Human activities causing this decrease, as quarrying, mushroom culture, sightseeing and banding of bats, have been discussed already in a preliminary publication (SLUITER and VAN HEERDT, 1957).

6. JUVENILE MORTALITY AND REPRODUCTION

Our estimates of the survival rate during the first half year of life are apt to be very inaccurate for two reasons. First, they are calculated from three estimates (adult survival rate, sex ratio and factor of decrease of the population), each of which may contain a considerable error. Secondly, they are based upon the assumption of constancy of the adult female survival rate during the year (*cf.* SLUITER, VAN HEERDT and BEZEM, 1956, p. 72), an assumption which is probably not realized. When using our estimates mentioned above for the estimation of juvenile surviving the possible errors of these basic data may accumulate and thus cause a very serious bias in our estimate of the juvenile survival rate.

Especially an error in the adult survival rate has very serious consequences. A negative error of 0.05 in this quantity introduces a positive error of 0.20–0.30 in $\sqrt{p(0)}$.

Consequently, we are not much surprised to find a value > 1 (which is, of course, impossible) in *M. myotis* and in *M. mystacinus* and a juvenile survival rate as high as 0.90 in *M. dasycneme*, which is obviously too high.

Finally, our estimate of juvenile surviving depends on the value of the reproduction rate. In the genus *Myotis* this rate is obviously one young per annum per female, the females of the first year-class excluded,

as the fertile females are known to ripen one Graafian follicle each year. In the genus *Rhinolophus* the evidence on reproduction rate is, however, insufficient to base an estimation of the juvenile surviving upon.

VI. LIFE TABLES

Life tables, derived from an estimate of the survival rate, provide, of course, no new information. They are still useful as an illustration of the consequences, which originate from the differences between the values of the survival rate, which were found.

TABLE X

Years after ringing	Survivors out of a 1000 ringed			
	M. daub.	*M. myst.*	*M. emarg.*	*Rh. hipp*
0	1000	1000	1000	1000
1	800	752	701	567
2	640	566	491	321
3	512	425	344	182
4	410	320	241	103
5	328	240	169	59
6	262	180	119	33
7	210	136	83	19
8	168	102	58	11
9	134	77	41	6
10	107	58	29	
11	86	43	20	
12	69	33	14	
13	55	25	10	
14	44	18	7	
15	35	14		
16	28	10		
17	23	8		
18	18			
19	14			
20	12			
21	9			

In tables X–XII we have restricted ourselves to *M. daubentonii*, *M. mystacinus*, *M. emarginatus* and *Rh. hipposideros*, because no evidence of ring mortality, biasing our estimate of adult survival rate, exists in these species. We have published similar life tables of *M. mystacinus* before. In the present paper, the different treatment of the data (*cf.* p. 513) results in a slightly different value of the adult survival rate in this species. Therefore, a correction of the life tables in our previous paper is needed here too.

1. SURVIVORS OUT OF A THOUSAND RINGED; LIFE SPAN

In table X, the expected numbers of survivors out of an initial number of 1000 full-grown ringed specimens, belonging to any year-class or combination of year-classes, are calculated by means of the values of the survival rate, as recorded in table IV.

In table XI, the life span, defined again as the largest number of years after which at least 10 individuals out of a 1000 ringed are still alive, is recorded.

Comparing the extreme cases among the investigated populations, it turns out that *M. daubentonii* may live up to 20 years, *i.e.* more than twice as long as *Rh. hipposideros*.

TABLE XI

Species	Life span	Expectation of life	Mean longevity
M. daub.	20	4.5	5.0
M. myst.	16	3.5	4.0
M. emarg.	13	2.8	3.3
Rhin. hipp.	8	1.8	2.3

2. EXPECTATION OF LIFE AND MEAN LONGEVITY

Expectation of life and mean longevity, as recorded in table XI, have been calculated by means of formula (25) on page 74 of our previous paper on *M. mystacinus*. The values of this quantity are remarkably high, if we take into account that the bats concerned here are very small mammals (body weight: 5–10 gram).

TABLE XII

Age-distribution in fractions of total winter populations (sampling caves combined)

Age in years	*M. daub.*	*M. myst.*	*M. emarg.*	*Rh. hipp.*
½	0.200	0.248	0.215	0.361
1½	0.160	0.186	0.169	0.231
2½	0.128	0.141	0.132	0.147
3½	0.102	0.105	0.104	0.094
4½	0.082	0.080	0.082	0.060
5½	0.066	0.060	0.064	0.039
6½	0.053	0.044	0.050	0.025
7½	0.042	0.034	0.039	0.016
8½	0.034	0.025	0.031	0.010
9½	0.027	0.019	0.024	
10½	0.021	0.015	0.019	
11½	0.017	0.010	0.015	
12½	0.014		0.012	
13½	0.011			

3. AGE-DISTRIBUTION IN WINTER POPULATIONS

In table XII, fractions of the total population are recorded for each year-class. Contrary to our previous paper the sexes are combined in this table, because of the difficulties in obtaining an unbiased estimate of the sex ratio (*cf.* p. 534).

Here again, the differences between the populations of the bat species concerned, are clearly illustrated. For instance, according to their survival rates, the first year fraction is as low as 20 % in *M. daubentonii*, but as high as 36 % in *Rh. hipposideros:* individuals of 9½ year old still provide 3 % of the population of the former species, but almost are lacking in the latter one.

VII. SUMMARY

Population statistics were studied in populations of *Myotis mystacinus*, *M. daubentonii*, *M. emarginatus*, *M. dasycneme*, *M. myotis* and of *Rhinolophus hipposideros* by means of the marking-recapture method.

In the *adult survival rate* no differences between individuals of different sex (table II), of different age, or hibernating in different caves (table III) have been found. The estimates of this rate in *M. myotis* (0.637) and *M. dasycneme* (0.667) are probably too low, as a consequence of ring mortality, and, therefore, no life tables of these species can be recorded. In the populations of *M. mystacinus*, *M. daubentonii*, *M. emarginatus* and *Rh. hipposideros* the following values of the adult survival rate have been found: 0.752, 0.800, 0.701 and 0.567 respectively. From these estimates *life span* is calculated as resp. 20, 16, 13 and 8 years, *mean longevity* as resp. 5.0, 4.0, 3.3 and 2.3 years. The *age distribution* during hibernation, calculated in these four populations, is recorded in table XII.

The specific differences between all these estimates are in accordance with the fact that the *population size* of *M. mystacinus* and *M. daubentonii* remained constant, while that of *M. emarginatus* and *Rh. hipposideros* decreased with about 10 % annually during the period of investigation (table VIII). A similar rate of decrease has been found in the populations of *M. myotis* and *M. dasycneme*.

In the *rate of capture* no differences between the sexes have been found, with the possible exception of *M. daubentonii* in the cave named Slavante (table II). In the latter species the data from Slavante suggest that males are more easily found than females, but this result is not obtained in the other sampling caves combined. Regional differences in the rate of capture are recorded in tables III and IV in *M. mystacinus*, *M. daubentonii* and *M. emarginatus*. Generally speaking, in both sexes the rate of capture is higher in Slavante than in the other caves.

In all cases, the *sex ratio* of the bat populations hibernating in the caves is found disproportionate in advantage to the males, except in *M. daubentonii*, where the reverse holds (table VII). This ratio has been found to be constant during the period of investigation, except in *M. mystacinus* and *M. daubentonii* in Slavante (table V). In three species the fraction males is found to be lower in Slavante than in the other caves combined (tables VI and VII).

The particularities of the sex ratio, mentioned above, suggest that males and females have different habits in choosing a quarter to spend the winter.

It turned out that calculation of the *juvenile survival rate* leads to highly unreliable results. Though satisfactory values as 0.499 and 0.697 during the first half year of life were obtained in *M. daubentonii* and *M. emarginatus* respectively, in the other *Myotis* species the value found for juvenile surviving is undoubtedly too high. In *Rh. hipposideros* the evidence on reproduction rate is insufficient to allow an estimation whatever.

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ANNUAL REPORT
OF THE ZOOLOGICAL STATION OF THE NETHERLANDS ZOOLOGICAL SOCIETY FOR THE YEAR 1957

Owing to advanced schemes for extension of the Zoological Station, of which more will be said later on, the year 1957 somewhat lacked a quiet atmosphere, but nevertheless promising results have emerged again from our investigations.

The investigation by Mr. J. F. W. NUBOER, Utrecht, mentioned in our previous annual report, was continued in 1957 with renewed efforts. The idea was to measure the spatfall of mussels *(Mytilus edulis)* during one or more tidal periods at different depths of water, at the same time taking into account the current velocity, temperature, salinity and amount of light at those depths, and the quantities of mussel larvae and young mussels of various sizes present in this water. Measuring the spatfall was made possible by the development of a satisfactory method in the previous years. It had been shown (see DE BLOK and GEELEN in Arch. Néerl. de Zool., 13, 1. Suppl., 1958) that threads of embroidery silk and other material are in great demand with settling mussels. Such threads, of a known length, were attached to wooden frames, which were suspended in the sea. Mr. NUBOER had made some improvements in the 1956-method, and, with the kind cooperation of the Physical Laboratory at Utrecht, he had constructed a simple photometer, while the Oceanographic Department of the Royal Netherlands Meteorological Institute took care of the current velocity measurements. We were still in doubt whether the numbers of mussels, settling in 1½–2 hours on 20 meters of thread would be large enough to provide reliable data about the actual settling of spat, especially as we intended to investigate separately the behaviour of young mussels, just out of metamorphosis, and that of slightly older young, which may settle for the second or third time after having left their original substratum. We therefore began, in April 1957, by estimating the number of mussellarvae found in a certain amount of sea water, and started the more extensive observations only after we were sure that sufficient numbers of mature larvae were present in the

plankton. The quantitative study of the plankton samples and the determination of the number and the size of the mussels on the threads hung out at sea was a terrific job, which at the beginning was carried out by Mr. NUBOER, but later on was taken over by Mr. DRAL, assisted by Miss STEINFORT SCHAAP. However, the results were well worth the trouble, and they even surpassed our wildest hopes. There were 2 hour periods in which as many as 300 mussels and over settled on one single thread. A substantial addition to our knowledge was the discovery that practically no spat settled at still water. The settling took place mainly when the current was strongest. Hitherto we had always assumed that, just as with the oyster, no settling would be possible with a strong current. Furthermore, many data were obtained about transportation and settling of younger and older stages at various current velocities. In this way we got valuable additional data to other observations, collected by Mr. LUCAS in 1950, which so far had partly been a mystery to us. Though we regret that it will be a long time before the data of Mr. NUBOER and the earlier ones of Messrs. LUCAS, ROOTH, VAN DONGEN and VAN HAAFTEN will have been worked out completely, we are now in a position to do so and the efforts of all those years have not been in vain.

Moreover, as a side-issue, valuable data were obtained about the reproduction of *Mytilus*, which led to the plankton investigation by Mr. DRAL being continued throughout the year. When we started in early April 1957 the first larvae were already there, while at the end of the year we still found young larvae. In fact the reproductive period has extended over the whole of the year after March, the largest number of young larvae being present in the period June–July. The development from new born larvae to the stage of metamorphosis took about 4–5 weeks in spring.

Mr. DRAL spent also much time to all kind of laboratory work, so that not much time was left for other subjects, the more so as he attended a histological training course of 2 months at the Zoological Laboratory of Amsterdam University. Still, he devoted some time to the study of the movements of the septa in the exhalation siphon of *Mytilus*, movements which are of importance in the regulation of the amount of water pumped. He also tried to investigate the influence of temperature on the lateral cilia of *Mytilus*, but he did not arrive at conclusive results.

Miss BOSCH and Miss STEINFORT SCHAAP spent much time in measuring jellyfish, caught in large numbers in Mr. CREUTZBERG's widemeshed plankton nets at the lightvessels Noordhinder and Haaks in 1956 and '57. By using these nets continually throughout the months of spring, when the catch is collected at the end of every low

and high tide, valuable data were obtained on the presence of the younger stages of *Cyanea capillata* and *Aurelia aurita*. Small young of *Cyanea*, born a month before at the utmost, were found to be present from the beginning of January to at least the middle of May; small young of *Aurelia* were caught from mid-February also to mid-May. This means that the production of ephyrae by the polyps of these species must have taken place from about mid-December to at least mid-April, a very long period indeed. However, *Cyanea* showed a definite peak of very young jellyfish from January 24th to March 28th. With *Aurelia* it is not well possible to say anything definite about the maximum time of production of jellyfish, as the sea around the light-vessels, far off the coast, is not its proper habitat. The young *Cyaneae* disappear from the surface water at a size of about 5 cm, when we assume their age to be 2 months at the utmost. Their disappearance certainly results from the fact that they are too heavy then to be carried upwards by turbulence, while at the same time they show no vertical movement of their own as yet. Such movements do not start before fine days in April.

A subject, which had several times been recommended to students, the study of which, however, presented so many difficulties that it had never been accepted so far, was chosen by Mr. Van Erp, Utrecht. It dealt with the possible influence of temperature on the strobilation of the polyps of jellyfish. Though the various species of jellyfish show rather large differences, we may say that, generally speaking, sexual reproduction takes place in summer, while the polyp generation strobilates (that is to say produces young jellyfish by asexual reproduction) in winter. The cycle as a whole is determined chiefly by the time of strobilation, because the young jellyfish grows up, becomes sexually mature and produces eggs (out of which, after a short larval stage, polyps develop) without any resting period. The polyp stage, however, does show a resting period, which, in a way, is terminated by its strobilation. One would therefore like to know which factor governs the period of strobilation. It seems likely that, as strobilation is apparently connected with a definite time of the year, either temperature or light is responsible for its beginning. There are some indications in literature that temperature indeed plays a part.

By keeping sexually mature jellyfish for some time in tanks with a weak water current, the bottoms being covered with shells etc., an abundance of polyps of *Chrysaora hysoscella* were obtained, and also smaller numbers of polyps of *Aurelia aurita* and *Cyanea capillata*. At the end of the year they were submitted to the influence of various temperatures. The investigation has not progressed far enough to say much about the results, but the influence of temperature on stoloniza-

tion and possibly also on strobilation seems to be considerable. It looks as if we had taken up a subject here, which will keep us busy for a number of years. The refrigerator, donated at the time by the Netherlands Organization for Pure Research, proved to be most valuable for this investigation. We applied for it especially with a view to this subject.

The investigation of the movement of shrimps in the Wadden sea, from shallow water to the gulleys, was continued in 1957 by Mr. J. J. Beukema, Groningen, who worked at Den Helder for 6 months. It was a continuation of the research by Mr. Glas and Mr. Heyligers, carried out in 1953 and 1954. The starting point were the questions: 1. How does the shrimp, visiting the sand flats at high tide, perceive that the water is going out again, so that it has to leave the banks?, and 2. What external factors does it use for orientation when leaving the flats? Mr. Glas established that a change in water pressure is not involved and that, obviously, the animals follow the diminishing head of water by perception of the water's surface. Mr. Heyligers gave those data a more solid foundation. Both did not quite arrive at the second question, the orientation problem, but it looked as if the animals not just left automatically with the current. After unsuccessful trials in the laboratory Mr. Beukema transferred the investigation to the field, where he discovered that the animals, when leaving the flats for the gulleys, cross the current which is going over the bank more or less parallel to the gulley. Of course this direction is opposite on the two sides of the gulley. Though the point is not quite settled as yet, Mr. Beukema thinks it possible that orientation is connected with the sun. If this is true, then there is certainly no question of a fixed direction in relation to the sun, like the one demonstrated by Papi and Pardi with *Talitrus*, but the direction would depend on the direction in which the animals first ascended the bank. Sun orientation would then be similar to the kind of orientation used by ants in spring.

In February, 1957, Miss A. J. Warburg, Groningen, wound up the investigation mentioned in our previous report. It dealt with the question whether the preference of nudibranchiate slugs for certain Coelenterate species is innate or acquired. It was thought to be possible to make the animals accept a certain Coelenterate species, which formerly they refused, by offering this species as sole food to the youngest stages. We hoped that in this way it might be possible to establish a stock which would prefer a species different from the normal prey.

From Miss Warburg's investigation it appeared that several hypotheses, which had been used as a starting point, did not hold good. To begin with, it is not true that as a rule *Aeolidia* refuses the sea anemone *Diadumene* as a food species; on the contrary, every individual accepts

Diadumene, but in a relation to *Metridium* of abt. 1 :7. So, all one could hope for was that by breeding experiments and offering *Diadumene* to the young animals the relation *Metridium*: *Diadumene* might be changed in the offspring. Secondly, *Aeolidia* turned out to have a rather long span of life as a larva, perhaps 8–12 days instead of just a few. This made the rearing of the young animals much more difficult than we had expected. Thirdly, it looked as if the young animals were rather defenceless against the sea anemones; the anemones tended to eat their supposed predators. Through all these difficulties the investigation yielded no positive results, but it had so many interesting aspects that it is to be hoped that it will be taken up again sooner or later.

The apparatus which will be used by Mr. De Blok to study the influence of lunar and tidal movement on the reproductive periodicity of marine animals was completed at last in 1957. On account of the important summary on lunar periodicity in marine animals, given by Mr. Korringa in Ecological Monographs 1947, Mr. Korringa and Mr. Verwey together applied for a grant to the Organization for Pure Research, in order to find out what factors ultimately induce periodicity. In his first year Mr. De Blok made himself thoroughly acquainted with the literature on the subject, and he gave a theoretical review about the way in which lunar and tidal movements apparently make themselves felt. In the second year he designed the apparatus. His idea to include in the investigation the whole complex of possible factors did not withhold the Netherlands Organization for Pure Research to bear its cost. In the third year the schemes were worked out and substantially altered, because it was decided to convert the mechanically produced rhythmic phenomena not by electric, but by pneumatic means into rhythmic environmental influences. The fourth year was wholly spent in building the complex apparatus, which was completed in the fifth. The total installation has cost about *f* 28 000.— on the part of the Organization for Pure Research and several thousands on our own part, while Mr. De Blok got at his disposal one of our seawater tanks and a dark cellar. In designing and building the apparatus Mr. De Blok has received valuable help from Mr. Duk, instrument-maker at the Zoological Laboratory at Leyden. We shall soon see whether our expectations about this apparatus will come true. Now that Mr. De Blok has been taken over by us from the Organization for Pure Research, we want to express our thanks to this Organization for the confidence and patience with which it has attended our work on this item.

The investigation by Mr. Creutzberg, also paid for by the Netherlands Organization for Pure Research, yielded some new results in 1957. In 1956 he found that the elvers, entering the Wadden Sea from the

North Sea in spring, use a mechanism by which they let themselves be transported passively during the flood and seek the bottom during the ebb. Obviously, the animals are able to distinguish between ebb and flood. In the surface water the catches during flood averaged over 3 times those during ebb, but the catches made when navigating with and against the current were always practically equal, which proves that transport is passive. These results were born out by additional evidence, from which it appeared that the migration takes place both at night and day, though in day time at a certain depth. Moreover, it appeared that the elvers concentrated in the inner part of the Wadden Sea not far from the sluices in the closing dyke of the Zuiderzee, obviously because they were hesitant to enter fresh water. In this area flood- and ebb-catches proved to be of the same order, which points to the animals oscillating there with the tide. Later in the season, from the last week of March onward, they entered the sluices. This happened one month after strong migration in the outer part of the Wadden Sea had started, nothwithstanding the fact that, with their ebb–flood-mechanism, they could have reached the closing dyke in a couple of days. Some years ago DEELDER had proved already that, long before they enter, the elvers are present in the sea outside the sluices.

Mr. CREUTZBERG noted once that the migration changed into the opposite direction, possibly under the influence of a cold northern wind. The catch during the ebb was 25 times higher than that during the flood; it was clear that the animals fled from the inner area of the Wadden Sea on that occasion. Mr. CREUTZBERG assumes that the direction of transport, both with flood and ebb, is determined by both temperature and salinity.

Mr. CREUTZBERG also tried to find data in favour of an urge of the elvers to migrate to a fixed point of the compass. To this end the animals were brought into a round tank, the rims of which sloped gradually upwards, thus allowing a clear view of the sky. The experiments did not yield any evidence in favour of the supposition that the animals would have a preferred direction of escape. Everything seems to indicate that it is exclusively the ebb–flood-mechanism, which determines the direction of migration. A short summary of the data obtained so far is to appear in "Nature" (see Vol. **181**, p. 857–858, 1958).

Finally, Mr. CREUTZBERG investigated whether there were any indications that the old eels—of which it is proved that they follow a fixed course over a long distance when leaving the Baltic—may be guided by the direction from which certain vibrations reach the animal. It would lead us too far to enter into this question here, but the outcome of the experiments does not exclude this possibility.

Mr. Dudok van Heel spent the first few months of 1957 in getting acquainted with the job he had taken over from Mr. Kristensen: buying animals from the fishing fleet and providing the Universities with material for study. After that he gave some time to his own line of investigation, viz. the perception of sound by seal and porpoise, a subject he was already interested in at the time he entered our service. He made use of a young seal, which had been brought up for 1 ½ years by Mr. De Haan, warden of the Texel Museum. The animal was trained to come and fetch fish at a source of sound. With a simple apparatus it could be established that the upper limit of hearing is over 16000 Hz and that the susceptibility decreases rather quickly under 100 Hz. It was not well possible to establish the perception of direction, owing to reflection of the sound in the stone basin. A beginning was made of training the animal to react to differences in pitch. We owe many thanks to Mr. G. J. De Haan, who, by his great interest and unremitting helpfulness, greatly stimulated the investigation.

At the same time Mr. Van Heel, together with Mr. De Haan, tried to find a means to get hold of live porpoises for the same investigation. Before the war this species was numerous in the Wadden Sea, but ever since it was found to have become rather uncommon. It is not likely that this decrease is connected with the loss of the Zuyderzee, because the dyke was closed in 1932 and porpoises were still numerous in 1940. However, roundabout 1940 the Zuyderzee herring disappeared and this may have had something to do with the disappearance of the porpoise. When it was clear that no *Phocaena* were available in our country Mr. Van Heel tried Denmark, where porpoises leaving the Baltic in winter are caught in the Little Belt. Part of a pond near the windmill of De Bol, Texel, a pond which is connected with the sea by a small lock, was fenced in. Anticipating our annual report of 1958 we may already state that at this moment a couple of *Phocaena* is swimming in this pond.

As in other years the acquisition of animals from the fishing fleet furnished a great many data, of interest either from a faunistical or an ecological point of view or in connection with migration studies.

Owing to the abnormally mild spring *Sepia officinalis*, the common cuttle-fish, was unusually early. The first animals came in at Den Helder 6th and 9th April. From 26th April they occurred in increasing numbers. The animals, moreover, were very numerous, also in the Easter Scheldt. If handled with care, the time of arrival and the number of *Sepia* is a good indication of the temperature in the sea in a certain year. In the late spring of 1956 the species was extremely late and hardly got as far as Den Helder, in the mild spring of 1957 the opposite did happen.

As to the occurrence in autumn of Atlantic migrants around Scotland it seems worth while to mention that not a single catch of *Mola mola* was reported. Mr. KRISTENSEN heard of 3 catches of ray's bream in late autumn. *Scomberesox saurus*, the skipper, stranded at the end of December in large numbers from Egmond to Vlieland. For the stranding of this species in Zeeland waters we refer to the contribution by WALRECHT in "De Levende Natuur", vol. **61**, pp. 32–34, 1958.

Mr. KRISTENSEN drew our attention to the fact that in the end of 1957 and in the beginning of January 1958 an enormous amount of young herrings *(Clupea harengus)*, about 12 cm long, together with a small number of sprats *(Clupea sprattus)*, were washed ashore alive near Egmond, where the animals were gathered by the basketful. Eye witnesses report that they did not get the impression that the herrings fled for predators. The observation may be of importance in connection with the regular strandings of species as bream, moonfish, skipper and others.

Mr. KRISTENSEN also drew our attention to the fact that a number of congers *(Conger conger)* were washed ashore after the cold spell in December. Most of them were still alive. The species cannot stand the cold very well; apparently it curls up then, probably to be washed ashore by the currents.

Other data are summarized in the list of special catches. Worth mentioning are 2 *Ctenolabrus rupestris* from near Den Helder in April, 2 *Scorpaena dactyloptera* caught off Texel and Terschelling in March, and 2 specimens of *Scylliorhinus stellaris*, the southern dog-fish, off Texel in September. Furthermore, Mr. DEELDER informed us that a specimen of *Balistes capriscus* (♂? 41 cm), captured at 53°2′N and 3°20′E, was brought in at IJmuiden on 29th October. It is now in the collection of the State Museum of Natural History at Leyden.

Mr. WESTENBERG continued to work out his population theory. He intends to arrive at an insight in known phenomena without the use of mathematical formulations. His paper on "The mechanism of fluctuations in populations and the ups and downs of an unrestricted fishery" (Vakblad voor Biologen", vol. **36**, pp. 41–53, 1956) on the suggestion of Prof. Umb. D'ANCONA (Padua) was published in an Italian version (Archivio di Oceanografia e Limnologia (Venezia), vol. **11**, pp. 47–61, 1957). His contribution to the summer course of 1957 is to appear in "Vakblad voor Biologen", vol. **38**, pp. 21–27, 1958. He hopes to extend his theories to more complicated population systems.

Before we pass on the the hydrographical work we may add that Mr. VERWEY, besides to other people's investigations, devoted his time to the compilation of a non-official report on the influence of radioactive waste water on life in the sea.

In 1957, as in previous years, hydrographical research, under the

Species	*Sex; Size (cm)*	*Locality (ST means buoy on the Silverpit-Texel route)*
MIGRANTS SUPPOSED TO HAVE ENTERED THE NORTH SEA VIA DOVER STRAIT		
Raia brachyura	♂ 45; 90; ♀ 41	Texel Hole
Raia montagui	♂ and ♀ 40–71	Tea Kettle Hole, Texel Hole, Terschelling Bank
Spondyliosoma cantharus	26; 22; 36; 41	N of ST 4, Westhinder
Trigla cuculus	♂ 23; ♀ 19.7	Texel Hole, off Callantsoog
Balistes capriscus	♂ ? 41	53°2′ N, 3°20′ E
Sepia officinalis		
Octopus vulgaris	nearly all of them alive	near L.S. Texel, Texel Hole
MIGRANTS SUPPOSED TO HAVE ENTERED THE NORTH SEA VIA THE NORTHERN ENTRANCE		
Scomberesox saurus		Stranded at Den Helder, Vlieland, Texel Egmond
Brama raii	53; 56; 56	Stranded at Scheveningen, Wijk aan Zee Egmond
NORTHERN SPECIES, RARE IN THE S. NORTH SEA		
Raia radiata	♀ 60	Texel Hole
Anarhichas lupus	38; 31	Pit 4 buoy, ST 4, Texel Hole
Scorpaena dactyloptera	16.5; 21	ST 4, Texel Hole
Hippoglossus hippoglossus	young	Callantsoog 13 m
Eledone cirrhosa	alive	N of Terschelling Bank
SPECIES, WHOSE DIRECTION OF MIGRATION IS UNCERTAIN		
Acipenser sturio	♀ 295	Texelstroom
Scylliorhinus stellaris	± 100, alive	Texel Hole
Scylliorhinus caniculus		Texel Hole, Terschelling Bank
Ctenolabrus rupestris	15.3; 15	near Den Helder
Loligo vulgaris		
Loligo forbesi		
Nephrops norvegicus		Terschelling Bank, Texel Hole, ST 3

Numbers per month											
J	F	M	A	M	J	J	A	S	O	N	D
1	1	—	—	—	2	—	—	1	—	—	—
17	—	1	2	3	4	1	1	3	2	7	—
—	1	—	—	—	—	—	—	—	—	3	—
—	—	—	—	1	1	1	—	1	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—
—	—	—	26	very large numbers		8	3	2	—	1	1
2	—	1	—	1	1	—	—	2	—	2	4
—	—	—	—	—	—	—	—	—	—	—	34
1	—	—	—	—	—	—	—	—	—	1	2
—	—	—	—	—	—	1	—	—	—	—	—
—	1	2	1	1	1	—	—	—	—	—	—
—	—	2	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	2	—	—	—
large numbers from 15 J on		3	2	2	2	—	—	2	2	7	32
—	—	—	2	—	—	—	—	—	—	—	—
—	—	—	1	17	5	30	—	5	—	—	—
—	1	2	—	2	1	very large numbers		25	—	—	2
2	—	1	—	—	1	4	—	2	45	3	—

direction of Mr. Postma, was mainly concentrated in the Wadden Sea, but additional data were collected about the IJsselmeer and the Zeeland and South Holland estuaries. When we add the observations obtained in a previous year in the Eems and Dollart area, we can say that we now possess a number of data concerning the more important Netherlands coastal waters. The point at issue is the chemical composition of the water and of the material in suspension. Special attention has been given to the question whether chlorophyll may be used as a standard for the amount of phyto-plankton present in the water. Mr. Postma has gradually become convinced that by far the largest part of the chlorophyll is present as dead matter. Comparable results in other areas point to the same conclusion. Even in the ocean an important part of the green plankton pigments is not confined to living matter.

Research in Zeeland was meant to give us some idea about an area which is shortly to be investigated more extensively by the Delta-Research Department of the newly erected Hydrobiological Institute. In so far as we may draw conclusions from one series of observations we may say that, just as with the Wadden Sea, suspended material from the North Sea enters the Zeeland estuaries. The organical part thereof probably furnishes an important contribution to the balance of organic matter in these estuaries. The influence of fluviatile matter is restricted to the area north of the Zijpe. The Wester Scheldt is enriched to a great extent by organic matter and minerals from the Scheldt (Antwerp), so that this area occupies a separate place in the complex of the Zeeland and South Holland estuaries.

The data collected in the IJsselmeer are concerned with the distribution of biologically important minerals in this area. Special mention should be made of the fact that the horizontal distribution of the various components, besides by the discharge of the IJssel and of the city of Amsterdam, was at the time of the investigations apparently determined by material from the dyke which is built to connect the island of Marken with the mainland. This influence seemed to be present all over the IJsselmeer, also as concerns e.g. the amount of chlorophyll and phosphor[1].

Mr. Postma again devoted much of his time to working out the chemical data of the Snellius expedition. The results of this investigation are shortly to appear in print. They lead to a clearer notion of the way in which various water masses enter the Indonesian area from the Pacific and of the changes in chemical composition of the water during the transport through the Indonesian Archipelago.

Mr. Duursma continued his investigation about the amount and

[1] See, however, Annual Report for the year 1958.

possible composition of dissolved organic matter. As mentioned in the previous report he has worked out analytical methods allowing the accurate determination of carbon and nitrogen in small amounts of dissolved organic matter. In this way he hopes to get some indication on the origin and decomposition of these materials.

In the past year Mr. Duursma investigated a number of samples from the Wadden Sea, the Eems and the IJsselmeer, with the intention to find out what changes may occur when salt and fresh water mix. Large amounts of organic matter in solution are carried into the Waddensea with the river water. So far, the results give no indication of a speedy alteration of this material, once in the sea. In view of its high concentrations in the sea, even at great depths, such an alteration was not to be expected either.

It is Mr. Duursma's intention to extend his investigations to the Atlantic Ocean, so that it will be possible to compare water from the coast and from the high sea. A preliminary investigation was made possible, since Mr. Duursma got the opportunity to join a trip of the German research vessel "Gauss" to the Norwegian Sea between Jan Mayen and the Norwegian coast. On this occasion water samples were taken to a depth of 3000 m. They confirmed the notion that in this deep water rather high concentrations of dissolved organic matter may be found. However, there were measurable differences along the vertical. Continuation of the research in open sea may therefore lead to important conclusions and this justifies Mr. Duursma's scheme for the investigation of a great number of samples which will be collected by German research vessels on the occasion of the International Geophysical Year, between the Azores and Greenland. In this connection special mention should be made of the cooperation rendered by the German Hydrographic Institute at Hamburg.

As to the work nearer home we mention a number of water samples from the harbour of Den Helder, which were tested for salinity on behalf of Rijkswaterstaat (Ministry of Roads, Canals and Dykes). We also cooperated with the same service on the movements of water marked with fluorescene. The observations were carried out to find the best place for discharge of the sewage from the town of Den Helder.

We should finally mention that Mr. Postma published two hydrographical papers: Size frequency distributions of sands in the Dutch Wadden Sea, in Arch. Néerl. de Zoologie, vol. **12**, pp. 319-349, and, together with Mr. P. Korringa: Investigations on the fertility of the Gulf of Naples, in Pubbl. Staz. Zool. Napoli, vol. **29**, pp. 229–284.

In the beginning of 1957 discussions were held with the Organization for Applied Research, which led to the Station offering accomodation to this Organization for research concerning means of combating fouling

of ships. Mr. De Wolf, to whom this investigation was entrusted, had at first found accomodation with the State Institute of Fishery Research at IJmuiden; however, this institute was cramped for space. Mr. De Wolf started working at Den Helder on March 15th. He intended to choose a species of barnacle as the chief animal for his experiments, hoping that he would be able to raise larvae throughout the year. So he set up a culture of barnacles on the one hand and on the other hand a culture of phytoplankton to serve as food to the larvae. Since nowadays it is very important for any marine biological station to possess phytoplankton cultures, the experiences of Mr. De Wolf are also of great value to the Zoological Station.

Among the other institutions with which the Zoological Station had connections especially the State Institute for Fishery Research at IJmuiden, inclusive its oyster laboratory at Wemeldinge, should be mentioned. The scientific staff of IJmuiden and Den Helder strengthen the ties by mutual lectures; investigations on the lightvessels are organized by the two institutes together, while for a time in 1957 Den Helder granted facilities to IJmuiden so that lobsters could be isolated for studying a disease of this species in Zeeland.

Relations with the Oceanographic Department of the Royal Netherlands Meteorological Institute at De Bilt are also good. This Department helped us repeatedly by lending an Ott current meter, even together with the man who worked it, and by lending the very valuable self-registering salinity and temperature meter built by the Institute. We are further on good terms with several services of the Rijkswaterstaat, an institute of much value to any marine institution. Further, we are much obliged to the Royal Netherlands Navy and the Pilotage at Den Helder for help on various occasions. A special word of appreciation is due to the crew of the lightvessel Texel for the care with which they worked the large plankton nets. Finally, we should express our most cordial thanks to the personnel working the sluices at Den Oever for their cooperation in the elver-investigations.

Just as in previous years the summer courses were given by the entire scientific staff, including the members appointed by the Organization for Pure Research. The number of students participating in these courses was 47, of which 14 came from Groningen, 3 from Utrecht, 2 from Amsterdam and 27 from Leyden. One of the courses was attended by a Belgian biologist, Mr. Ph. Polk from Blankenberge. In addition, Amsterdam gave a private physiological course, conducted by Mr. Punt, with the assistance of Mr. Kristensen and Mr. Parma, while the Free University at Amsterdam gave a short course for 22 of her own 1st and 2nd year biologists, and Utrecht University did the same for 37 people.

The total number of man days spent in 1957 at the Zoological Station by individual investigators and course participants amounted to 1140; the average for the years 1947–1957 is 1122.

The Dutch visitors to the Station included a group of biophysicists of the Physical Laboratory of Groningen University, under the guidance of Prof. H. DE VRIES, the participants of an excursion to Den Helder and Texel of the Royal Netherlands Botanical Society, and the members of the section for Ecology and Phaenology of the Royal Society for Agricultural Science.

Foreign visitors to the Zoological Station in 1957 were Mr. and Mrs. BRODIE, Victoria University, New Zealand; Mr. and Mrs. DAWSON, New Zealand Oceanographic Institute, Wellington, N.Z.; Mr. and Mrs. ORR and Miss Sheina M. MARSHALL, Marine Biological Station, Millport, Scotland; Mrs. M. KALK-THOMAS, University of the Witwatersrand, Johannesburg, South Africa; Messrs. K. MASAYASU and H. TOSHIO, Tokyo, Japan; Mr. V. LEPETIC, Dubrovnik, Yugoslavia, who worked for a long time at IJmuiden.

Under the heading Building we should say a few words about the plans for extension of the Station, to which much time was devoted in 1957. In the spring of 1957 the council of the Society submitted to the Government a scheme for considerable extension of the scientific and technical staff of the Zoological Station which, starting 1958, would have to be realized in about 15 years. It would involve the building of additional quarters or a new institute. This plan was accepted in essentials by the Ministry of Education, Arts and Sciences with the consequence that a lot of conferences and other work had to be done by some Government departments, the Society's council and the Zoological Station itself. The main question was whether, in case a new building was found to be necessary, the Zoological Station should stay at Den Helder or not. The answer has been that Den Helder offers many favourable conditions, but that the site suitable for a new Zoological Station will be found with great difficulty or not at all. The schemes may be worked out further in 1958. In view of this uncertainty no alterations to the building and to the students' lodge were undertaken in 1957.

The library continued to expand, especially as concerns the periodicals. Although the allowance was raised to *f* 1100.— we regret to say that the budget was again exceeded, this time by *f* 600.—. We gratefully remember the contribution of the Netherlands Physical and Medical Congress, which allowed us to procure Vinogradov's The elementary chemical composition of marine animals. The State Institute for Fishery Research placed at our disposal a considerable number of duplicates, which made it possible to fill up some gaps in

our library. We are also much obliged to the Fisheries Research Board of Canada for sending us a collection of old issues of several of their periodicals. Our exchange relations with the Sovjet Union begin to take shape again after a long interruption. Last year (1956) the contact with Tartu was re-established and in the present year the Lenin-library in Moscow took the initiative for exchange.

In the previous annual report we mentioned already that in the winter of 1956-57 the Max Weber got a new engine-block. Consequently the vessel was out of service until 15th March and during that time we used a ship hired at Harlingen: the Ha 42, which, together with its skipper Dirk Bos, has helped us out. The number of working days of this ship was 28, that of the Max Weber amounted to 87, so that 113 days were made in all. At the end of '57 and the beginning of '58 the wheel-house of the Max Weber was well enlarged. There is now room for some instruments and more room for unrolling charts, writing and other work and for the men. The shape of the ship has in fact improved and the navigability in rough seas has not noticeably changed. The alteration was made possible by a separate grant of *f* 3100.— by the Department of Education, Arts and Sciences, which also furnished the costs of reparation and of the hire of the Ha 42.

The provision with sea-water of the Zoological Station, which since the autumn of 1951 was looked after by a private person, in November 1957 was taken over by the Royal Netherlands Navy. About every other day a ship delivers a quantity of some 50 m³ of sea-water, and since the skipper tries to get water of a high salinity the provision leaves nothing to be desired.

There were not many changes in the personnel, only the analyst Mrs Peeters-Planken resigned on February 1st. Appointed were 2 other analysts: Miss C. M. Bosch and Miss. S. Steinfort Schaap, on 1st Jan. and 1st Febr. respectively. On March 15th Mr. Prins celebrated 25 years of service with the Zoological Station. Starting 1 July Mr. Postma was appointed substitute director.

As mentioned above Mr. Duursma took part in a 3 weeks trip with the German research vessel "Gauss", stationed in Hamburg. On this occasion Mr. Duursma also attended the meeting of the International Council for the Study of the Sea at Bergen, Norway.

In the company of Mr. Korringa, IJmuiden, and Mr. Windemuller, physician at the Netherlands Reactor Centre, Petten, Mr. Verwey visited some English institutes carrying out investigations on the influence of radioactive material on marine life. The visit was connected with the above mentioned report on this subject to the President of the National Health Board.

Mr. Verwey used up part of his holidays by visiting German marine

biological and fishery laboratories: the "Biologische Anstalt" at Hamburg and at List on the island of Sylt, the "Institut fur Meereskunde" at Kiel, the "Deutsche Hydrografische Institut" at Hamburg, the "Bundesforschungsanstalt für Fischerei" at Hamburg with its branch office at Cuxhaven, the "Institut für Meeresforschung" at Bremerhafen. Thus he was in a better position to compare the German institutes with the French and English ones he visited in previous years. He also visited the Hydrobiological Station at Plön, Kramer's Institute of the "Max Planck Gesellschaft" at Wilhelmshaven, and the fine aquarium of the Institute "Senckenberg am Meer" at Wilhemshafen.

Mr. Creutzberg also visited some institutes abroad during his holidays, viz. the Fishery biological Institute at Drottningholm, Sweden, the Station Kristineberg on the Swedish West Coast, and the Max Planck Institute as well as the institute Senckenberg am Meer at Wilhelmshaven.

The receipts from study materials in 1957 amounted to *f* 9645.42, while *f* 4467.38 was spent on the acquisition of animals and *f* 1768.26 on preserving liquids, wicker bottles and other materials for expedition, so that the expenditure amounted to *f* 6235.64. This makes a balance of *f* 3409.78, a somewhat higher amount than before as a consequence of our attempts to make this part of the work somewhat more self-supporting. In fact, the work practically represents a full-time job for one of our men, and it is therefore rather costly. We intend in the near future to enter into special demands from university laboratories for histological or other more specialized purposes.

The total costs of exploitation, excluded the grants of the Netherlands Organization for Pure Research, amounted to *f* 130000.—. When we add to this the costs for the apparatus of Mr. De Blok amounting to *f* 28000.—, the total expenditure for 1957 amounted to *f* 160000.—. We are much obliged to the Government for the considerable support now given to marine biological research, and it may not seem altogether realistic that at the same time we plead for the Station's extension. However, when taking a broad view of the matter, we must be aware of the fact that also this field of research should stand on a high, that is to say on an internationally recognized level. This is only possible if the ever increasing specialization in this branch of science also finds expression in our country, a state of affairs involving considerable sacrifices anyhow. It is reassuring that the Government does its best to promote this development.

Den Helder, February 1958 J. Verwey

ANNUAL REPORT
OF THE ZOOLOGICAL STATION OF THE NETHERLANDS ZOOLOGICAL SOCIETY FOR THE YEAR 1958

In this report on the research carried out by the Zoological Station in 1958 we intend to break with the custom founded in post-war years, viz. to open the report with the results of the ecological investigations on shell-fish. For years on end such investigations have played an important part in the whole, but gradually other aspects of biological research have come to the foreground and it seems reasonable to start with those items now.

In the 1957-report we gave a survey of the progress made by Mr. De Blok in the years 1955–57, when he designed and built his apparatus to study lunar and tidal influences on the reproductive periodicity of marine animals. In 1958 Mr. De Blok, until then employed by the Netherlands Organization for Pure Research, took up his post with the Zoological Station, and this was also the first year in which his experiments were definitely started. The point at issue was the influence of duration, intensity and shifting of the moonlight, together with the influence (varying with the tides) of intensity of sunlight and of pressure, on the reproduction of the mussel *(Mytilus edulis)*, the oyster *(Ostrea edulis)*, the sea-urchin *(Psammechinus miliaris)* and the polychaete worm *Spirorbis borealis*. The reproduction of the animals was checked by stating the presence of eggs or larvae in the outlet of the aquaria.

This check, carried out three times a week, yielded much extra work for Mr. De Blok and Miss Steinfort Schaap at the times when eggs were delivered. The animals were kept in slowly running water with aeration. The sea-urchin was fed mussel-flesh and sealettuce *(Ulva lactuca)*, the other animals got the chrysophyce *Phaeodactylum tricornutum* as food, which was obtained from a culture put up by Mr. De Wolf in connection with his *Balanus*-work, which made part of his investigations on the fouling of ships carried out at Den Helder, on behalf of the Netherlands Organization for Applied Research. Quite contrary to our expectations the oyster, fed with *Phaeodactylum*,

produced large numbers of larvae, while the mussel, which as a rule is not very particular in its wants, did not deliver any eggs at all. The sea-urchin did, but very irregularly. *Spirorbis* did reproduce, as there appeared new animals along with the old ones, but it seemed that we had not got hold of the right technique to catch the larvae.

So the only results which allow further elaboration are those of the oyster and they point to the existence of a periodicity of 28 days, notwithstanding the fact that the lunar month to which Mr. De Blok exposed his animals had 34 days. If this periodicity of 28 days is reliable it is not linked up with the moon or the actual tide in the sea, so that it might be likely that the animals experience an inherent periodicity of 28 days, which, however, as concerns the times of appearance, has shifted with regard to the lunar and tidal cycle outside the laboratory. In how far this shifting is due to the environment as it was offered in the experiment we cannot say as yet; so far there are no certain indications that the circumstances of the experiment have influenced reproduction. If the existence of an 28-days period should be confirmed in future, this will be an important additional result of the experiments.

In the past year Mr. Creutzberg, employed by the Organization for Pure Research, continued his investigation on the orientation of the elver, in which an important stage was reached. In the months of February to April a number of further data were collected by nightly fishing, completing the picture of the migration in the sea. It is now clear that the animals reach the inland waters in spring by passive transport during the flood, while they stop this transport during the ebb. This movement may be reversed by a drop in temperature, while it is probably a combination of temperature and salinity that decides on the question up to where the animals enter brackish water. In this area they let themselves be transported up and down with flow and ebb until (possibly not before many weeks have passed) the time has come to enter fresh water.—While these outdoor data were collected by our skipper, Mr. Creutzberg himself tried to prove by experiments that changes in salinity and temperature in the course of flood and ebb are indeed used for discrimination between the tides. To this end he designed an apparatus in which every current velocity and change in temperature or salinity might be applied. The outcome of this experiment meant a great disappointment, since nothing showed that the animals could distinguish flood and ebb. Later experiments indicated that his starting point had probably been wrong, because ebb- and flood-water are apparently not distinguished by temperature or salinity, but in another way. It looks as if Mr. Creutzberg may be able to wind up a substantial part of his elver-investigations in 1959 and

publish the results. The data are an important addition to our knowledge of the migration-mechanism of species with passive transport into a certain direction.

During his work in 1957 Mr. CREUTZBERG obtained data indicating that the shrimp *(Crangon crangon)* has an ebb-flood mechanism similar to that of the elver, enabling his transport into a certain direction (towards and away from the coast). A closer study of this question was taken up as a graduation subject by Mr. STERK, University of Utrecht. He found that shrimps leave the Waddensea by passive transport during ebb and enter it in the same way during flood. Just as with the elver there are apparently all sorts of transitions between transport into one and transport into both directions and this decides whether the animals stick to a certain area or not. The crucial point is certainly the combination of temperature and salinity in the area concerned, but this does not necessarily imply that ebb- and flood-water are distinguished by temperature and salinity.

Especially since HAVINGA's investigations (1930) we know that there are differences in the time of departure and of return of juvenile and adult shrimps and that the ♂♂ and the sexually mature ♀♀ go farther out in winter than the juvenile ♀♀. The return migration of the adult ♀♀, starting with the third decade of March, was very conspicuous. They were extremely late as a consequence of the late spring. It is remarkable that the relation between the nubmers of juvenile and adult animals during one and the same period is approximately the same with flood and with ebb. All this makes the whole picture very complicated. In autumn the total numbers are large with ebb, in spring they are large with flood. As the migration season proceeds the relation juvenile adults changes, but within any given period it is the same with ebb and flood. This would probably mean that part of the animals, transported outward with the ebb current, come back with the flood. Finally there is a difference in the requirements of ♂♂ and ♀♀, which makes the picture even more complicated, while the intricate hydrographical conditions off the tidal outlet of Texel, as proved by DIETRICH's studies (1953), probably greatly influence the movements within the sea.

As the next item of our report the investigation by Mr. DUDOK VAN HEEL be mentioned. As already stated in the previous report he was interested in the perception of sound by the seal *(Phoca)* and porpoise *(Phocaena)*. Together with Mr. DE HAAN, warden of the Texel Museum, he tried, in December 1957 and January 1958, to obtain a number of porpoises with the help of Danish fishermen near Middelfart in Denmark, where the animals pass in substantial numbers during autumn migration from the Baltic to the open sea.

Nineteen animals in all were caught there. Three specimens, and later yet another five, were taken to Holland by road. One of these stayed alive from the end of January till the 6th of June in a brackish water pond on the island of Texel. With this animal a series of observations were carried out to test the accuracy of sound location under water. It was taught to come and fetch a fish at one of two underwater sources of sound and fortunately it could also be taught not to come if no signal was given, so that its reactions to sound could be properly studied. Experiments proved that the animal was able to locate the direction of the signal under water, as long as the angle between the two signals was at least 16°. This angle is probably dependent on the frequency of the sound. A short communication on this subject is shortly to appear in Nature (see Vol. **183**, p. 1063, April 11, 1959). The work was subsidized by the Netherlands Organization for Pure Research, by Mr. DEN HERDER at Harderwijk, the Laboratory for Comparative Physiology of Utrecht University, and the Linnaeus Fund in Amsterdam.

In the 1957-report we also mentioned Mr. VAN ERP's research on the influence of temperature on strobilation and the formation and fission of ephyrae of jelly-fish polyps. The investigation exceeded the original scheme because, along with strobilation, stolonization of the polyps and the formation of so-called podocysts played an important part. Essentially, stolonization is the formation of buds, followed by the growth of stolons. Podocyst-formation is the development of encysted groups of cells which after some time may grow into new polyps. One might call the podocysts encysted buds which, after a resting period, are capable of revival. Strobilation is the well-known formation and subsequent fission of ephyrae which develop into the sexually mature jelly-fishes. In summer this jelly-fish produces eggs, out of which planula-larvae develop. Very quickly the latter grow into polyps, which may reproduce either by stolonization of by the formation of podocysts, and at last may give off ephyrae. Because the development of ephyrae is confined to a fixed time of the year it seems obvious that either light or temperature may govern this periodicity. The data so far obtained seem to indicate that light has no influence, but that temperature has; besides, there is also something like an autonomous periodicity. The polyps of *Chrysaora hysoscella* only showed strobilation above 8°C, but, also when kept at constant temperatures, not before mid-April; the polyps of *Cyanea capillata* showed strobilation at constant temperatures of 3, 6, 9 and 12 C, especially in midwinter with the lower, at the end of winter with the higher temperatures. The number of strobilating polyps increased with higher temperatures; also the strobila delivered

more ephyrae and in less time. So the jelly-fish production is then greatly increased. It must be kept in mind, however, that what we call higher temperatures here are in fact still low temperatures, viz. 9–12°C.

In Mr. VAN ERP's experiments stolonization played a large part with *Aurelia aurita*, of which one polyp may give rise to an offspring of tens of polyps in a few months time. It played no part at all with the two other species, *Chrysaora hysoscella* and *Cyanea capillata*, of which stolonization is mentioned in literature.

Podocyst formation played an enormous part with *Cyanea capillata*, for which it was not described before. According to Mr. VAN ERP it is quite a normal way of asexual reproduction with this species. Podocysts were also observed with *Aurelia* and *Chrysaora*. It looks as if podocysts occurred especially at the higher of the temperatures used (9–12°).

The polyps of *Aurelia* and *Cyanea capillata* can stand lower temperatures well, those of *Chrysaora* cannot. This is in accordance with the southern distribution and the late appearance in the season of the lastnamed species.

It is very much to be hoped that these investigations, with their many interesting aspects, will be taken up again by other students. There are not many facts known in this field of research.

Mr. H. L. DE BEAUFORT, who, in 1956, had been studying the causes of the development of one of the colour patterns of *Sepia officinalis*, the common cuttle, returned to Den Helder in 1958, hoping to make this work the subject for a doctor's thesis. The experiments have not yet yielded sufficient data to summarize the results, but a few words may be said on the keeping of the animals in question. When working with *Sepia* the main difficulty is the chance of injuries. Though now we have special basins available, measuring 5 × 1 meter, they hurt themselves so often when swimming backwards that they are unfit for experiments after three months at the utmost. It would be better to use much larger basins to prevent these injuries, but this also has its drawbacks, because it is not possible to keep more than one animal in one tank, as they maul each other badly. It was also tried to rear young *Sepias* from the egg, but we discovered too late that the water was undersaturated in respect to oxygen. A rather large number of young hatched, but they all died before they were 3 cm at the utmost, probably because the oxygen content of the water was somewhat too low. Perhaps, however, other factors, a.o. too low a temperature, also played a part. When still very young the animals already took *Mysis* and small shrimps.

On the suggestion of Prof. WOLVEKAMP, Mr. OSSE of Leyden University carried out a tentative research on the respiration of *Cancer pagurus* with various oxygen pressures in the environment. The intention was

to find out whether the intake of oxygen depended on the oxygen pressure, and, if so, in how far this dependence was connected with a small diffusion velocity of the epithelium of the gills, as was found by REDMOND for some American crustaceans, or whether it was due to the absence of any regulation of the oxidation within the tissues. The time could not be found, however, to determine the oxygen pressure in the blood, as would have been necessary to answer this question. Some attention was paid to the relation between oxygen pressure and body size.

Mr. DUBBELDAM, also of Leyden University, visited Den Helder to study the influence of various current velocities on the chances of survival of the hydroid polyp *Hydractinia echinata*. In coastal waters this species occurs mainly on the shells inhabited by the hermit crab *Eupagurus bernhardus* and seldom on shells still inhabited by gastropods. This must be because *Eupagurus*, which wanders about quite a lot, makes the water move around the shell, thus helping *Hydractinia* to get rid of settling silt. Where *Hydractinia* occurs in coastal waters on an immobile substratum there is either a strong current or the water is sufficiently clear. Now, our idea was to use recently settled young colonies and to measure their growth as an indication for current sensitivity. This meant we had to raise young colonies, as had been done by Miss SCHIJFSMA (1934). The whole scheme went wrong, however, since Mr. DUBBELDAM obtained only 2 male colonies in 200, all the other colonies being female. He found that in Woods Hole in 1894 Martha BUNTING stated an excess of ♂♂ in June and of ♀♀ in July. This was attributed by her to a change in external conditions. It is thought to be possible that the species is subject to a periodical change of sex and it might be worth while to set up further investigations. Mr. DUBBELDAM's observations with older colonies seemed to indicate that a one-way current was less favourable than an alternating one. Moreover, it seemed that a one-way current of 16 cm/sec was less favourable than a weaker one.

By order of Mr. KORRINGA and on behalf of the State Institution of Fishery Research Mr. H. OBREEN, Utrecht University, investigated the influence of current velocity on the food intake of the oyster. This research was started in the laboratory at Wemeldinge and continued afterwards at Den Helder. The results point to an unfavourable influence of the stronger currents (30 cm/sec) on the food intake. The experiments were carried out in the apparatus designed by Mr. CREUTZBERG. Food was provided in the form of *Phaeodactylum*, reared by Mr. DE WOLF, and the food intake was deduced from the increase in weight of young oysters, weighed under water in the manner described by HAVINGA (1928).

Mr. DEN HARTOG entered service with the Delta Research Department of the Hydrobiological Institute and spent the first months of his

employment at Den Helder. We will report on his work further below.

When, in the beginning of this report, we said that we did not start new investigations on shellfish, this does not mean that we dropped the subject, on the contrary. The study of the numbers and size of the mussel spat present in plankton, which was started by Mr. NUBOER in April 1957, was continued by Mr. DRAL, who studied water samples, collected at least twice a month until May 1958. New larvae turned up in the plankton in all months from April to November. Apparently, new animals were even born in January (1958). On the other hand no larvae were present at all in mid-May 1958, undoubtedly due to the cold spring. It is clear that 1957 has been an exceptionally good year for the production of spat.

Counting and measuring the mussel larvae from the water samples collected during Mr. NUBOER's investigation also took up a lot of time. They were meant to give an idea about the numbers present in the water as compared to the numbers settled on frames hung out at various depths in the sea. This work was done by Mr. DRAL, together with Miss STEINFORT SCHAAP and Mr. DE VRIES.

Finally, we must mention that Mr. DE BLOK worked out the data collected by himself and Miss GEELEN in 1952, on the conditions required by the mussel when settling on thread-like substrata. This publication forms part of the Supplement to part 13 of the Archives. In addition to the requirements of the mussel spat during settlement also the growth of the animals is dealt with. The plankton data for 1957 also yielded some data on growth, especially during the period preceding settlement. In brief, the results come to this. Spat of 100–260 μ showed an increase in length of 2.5% on an average and 4.5% as a maximum per day in mid-April. End May 1952 young mussels of 400–1600 μ showed a maximum growth of 8%, decreasing in the case of the bigger animals to 5%. Without further data we cannot say in how far the differences between spat and already settled mussels are due to the differences in temperature between mid-April and end May. Reliable data of this kind are scarce.

Continuing his former speculations Mr. WESTENBERG developed a theory on the cybernetics of populations, in which for the time being he did not include quantitative speculations. It served as a contribution to the summer course. We may add that his recent summary on the development of fishery theories, of which a Dutch, French and German version existed, unexpectedly turned up in a Russian translation as a separate publication. His contribution to the summer course of 1957 which, as mentioned in the previous report, was published in the "Vakblad voor Biologen", appeared in Italian in Archivio di Oceanografia e Limnologia, Venezia, Vol. **11**, p. 265–273.

Finally, we may add that Mr. VERWEY gave a summary of our knowledge of the orientation of aquatic animals as compared to that of birds, which appeared in the above supplement of part 13 of the Archives. He devoted much time to the study of the literature on the influence of radioactivity on life in the sea, and, moreover, worked up the results of the investigations carried out between 1920 and 1950 in the Zuyderzee and IJsselmeer into a lecture on the occasion of the Commemoration Day of the Leyden University.

As usual this report gives a list of species of which the occurrence is interesting from a distributional point of view or for other reasons. Items worth mentioning are 5 specimens of the lesser fork-beard, *Raniceps raninus*, from the months of spring, 4 *Blennius gatterugine* from December, 1 *Mullus surmuletus* from January, 1 *Solea lascaris* from June, all pointing to a southern influence in 1958.

Further, we heard of at least 8 specimens of the thresher, *Alopias vulpes*, all caught in September off the Dutch cost by pilchard fishermen. The sharks measured from about 1.5 to 4 metres.

In autumn *Lepas anatifera* were numerous on floating material in the southern North Sea. Mr. DEN HARTOG told us that at the time many algae of southern origin occurred with southern animal species attached, so, evidently, the goose barnacles had reached the North Sea by the Straits of Dover. It is well known that the distribution of *Lepas anatifera* is greatly influenced by strong and prevailing winds from a certain direction. The species is not indigenous in the southern North Sea.

As to the species reaching us by the northern route, 14 specimens of the skipper, *Scomberesox saurus*, were brought in, of which 2 were caught at sea near Den Helder and the others washed ashore, all in January. Further 2 specimens of ray's bream, *Brama raii*, were brought in, one washed ashore near Callantsoog, the other one caught West of Texel. One specimen of the squid, *Illex illecebrosus*, was found on the beach near Egmond in April. All three species belong to the group of species that are known often to strand alive.

A specimen of the flatfish *Zeugopterus punctatus* was caught in January off Texel, a specimen of *Maurolicus mülleri* in the Texelstroom in March.

Hydrographical work was carried out by Mr. POSTMA and Mr. DUURSMA as before, while, moreover, the Zoological Station extended hospitality to Mr. HOEKMAN, who joined the Delta Research Department of the Hydrobiological Institute as a chemist-hydrographer.

Research in the Wadensea was a continuation of the work of previous years, while data were again collected in the IJsselmeer, the Delta-region and the large rivers.

Species	*Sex; Size (cm)*	*Locality (ST means buoy on the Silverpit-Texel route)*
MIGRANTS SUPPOSED TO HAVE ENTERED THE NORTH SEA VIA DOVER STRAIT		
Petromyzon marinus	72; 77	Locks Den Oever; off Falga; off Cocksdorp (Texel)
Raia montagui	♂♂ and ♀♀ 40–99	Off Falga; ST 3; Texel Hole; Terschelling Bank
Raia blanda (brachyura)	living	Off Falga
Squatina squatina	♀ 67	Terschelling Bank, 32 m
Raniceps raninus	living ± 7	Off Den Helder; Waddensea
Mullus surmuletus	26.5	Texel Hole
Spondyliosoma cantharus	38	Texel Hole
Blennius gattorugine	living	Off Texel
Trigla cuculus	♂♂ and ♀♀ 25–31	Texel Hole
Solea lascaris	27.5	Texel Hole
Sepia officinalis	♂♂ and ♀♀	Off Petten, off Den Helder, Molengat, Terschelling Bank, Tea Kettle Hole, Black Bank
Octopus vulgaris	10; 11; 13; 13	Texel Hole
MIGRANTS SUPPOSED TO HAVE ENTERED THE NORTH SEA VIA THE NORTHERN ENTRANCE		
Scomberesox saurus	5 ♂♂ and 9 ♀♀ 34–39	Stranded near Den Helder; Molengat, Texel Hole
Brama raii	♂ 53; ♀ 53	Texel Hole; Stranded at Callantsoog
NORTHERN SPECIES, RARE IN THE SOUTHERN NORTH SEA		
Anarhichas lupus	54	10′ ONO ST 2; Black Bank
Eledone cirrhosa		20′ NW Texel Lightvessel; 2′ N Molengat 60′ NNW Den Helder; Terschelling Bank
SPECIES, WHOSE DISTRIBUTION IS UNCERTAIN		
Alopias vulpes	158–400	Off IJmuiden, Scheveningen, Zandvoort
Maurolicus mulleri		southern Texelstroom
Spinachia spinachia	10.7	Den Helder (seaw. side New Harbour)
Ctenolabrus rupestris	8; 9; 12.3 living	Westgat (Haaks grounds); Molengat
Zeugopterus punctatus	16	Texel Hole
Illex illecebrosus	♂ ? 36.4	Stranded at Egmond
Loligo vulgaris	♂♂ and ♀♀ 11–28.5	Texel Hole, off Callantsoog, Haaks grounds
Loligo forbesi	♂♂ and ♀♀ 11–35	Texel Hole, Terschelling Bank

Numbers per month											
J	F	M	A	M	J	J	A	S	O	N	D
—	—	—	1	1	—	—	—	3	—	—	—
6	3	1	—	1	—	1	—	1	1	1	6
—	—	1	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—
1	—	4	2	1	—	—	—	—	—	—	—
1	—	—	—	—	—	—	1	—	—	—	—
—	—	—	—	—	—	—	—	1	—	—	—
—	—	—	—	—	—	—	—	—	—	—	4
—	—	—	—	5	6	—	1	—	—	—	—
—	—	—	—	—	1	—	—	—	—	—	—
—	—	—	—	59	48	23	231	169	17	—	—
4	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	1	—
—	—	—	2	—	—	—	—	—	—	—	—
—	1	1	3	—	—	1	—	—	—	—	—
—	—	—	—	—	—	—	—	8	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—
—	—	3	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	12	?	?	?	?	?	?	2
—	—	—	—	2	7	46	?	?	>14	>2	1

In the previous annual report attention was paid to the form in which chlorophyll occurs in coastal water. More arguments were collected in support of the assumption that by far the largest part of the chlorophyll is present in dead matter. It bore out the view that this material is only of minor value as a measure for the living phytoplankton. In certain cases, however, chlorophyll appeared to be useful as a special characteristic of that part of the suspended material of which it is a component. Mr. POSTMA used a.o. the chlorophyll percentage of the total amount of suspended organic matter in the Dollart region to demonstrate that this matter is for the greater part brought down by the river Eems and not formed on the spot or brought in from the North Sea. He wrote a paper on this subject, which is to appear shortly, together with contributions of others studying various problems in the Dollart-Eems estuary.

In connection with previous investigations measurements were carried out near Ameland concerning the way in which materials in suspension are held in the Waddensea. At the time Mr. POSTMA set up a theory, which afterwards was extended by Mr. VAN STRAATEN and Prof. KUENEN of Groningen University. In this theory the sedimentation velocity of the material in suspension plays an important part. It is still too early to say anything on the data collected near Ameland, except that an important fraction of the suspended silt settles more slowly than was originally supposed.

In the course of 1958 Mr. HOEKMAN and Mr. POSTMA made a number of trips to the Delta region with the Max Weber to investigate the distribution of salinity and silt and of various biologically important components of the water in different parts of the Zeeland waters. In the previous annual report the purpose of this investigation was shortly mentioned.

The IJsselmeer data were collected at the same places and in the same season as in the previous year. The purpose was to find out in how far the distribution of biologically important elements in this area might be disturbed by material from dyke building and dredging, as was supposed in the previous annual report. The disturbing effect turned out to be substantially lower than was supposed on account of the previous observations and hardly to play a role at all. We may assume that the observed horizontal distribution of the various components does represent the situation as it would be without human interference. It is remarkable how far eutrophy has progressed in the area west of the line Enkhuizen–Lelystad, where the concentration of nutritious matter exceeds that of the rivers. At the same time the water leaving the sluices is poorer in nutritious components than that which is brought in by the river IJssel.

Mr. DUURSMA, who in 1958 was still employed by the Netherlands Organization for Pure Research, again devoted much of his time to preparatory work, and, lateron, to the study of water samples collected in 1958 by the German research vessels "Gauss" and "Anton Dohrn" on his behalf. These vessels made two trips, one in spring (March/April) and one in autumn (August/September). Mr. DUURSMA determined the carbon, nitrogen and phosphor content of the organic material in solution of some hundreds of samples in a transect from the southern point of Greenland to the South. These determinations took up a lot of time, and a large part of the next year will be taken up by working out the results. The description of the method has now got ready, however.

The concentrations of dissolved organic carbon, and therefore also of dissolved organic material, present in the open ocean, turned out to be much smaller than former investigations—especially those by KROGH (1934)—had led us to believe. The carbon content averaged about 0.5 mg/l, which amounts to a content of dissolved organic matter of somewhat over 1 mg/l. Furthermore, it appeared that—also contrary to former assumptions—there may be substantial differences between the carbon values in various places, while, moreover, in the surface waters important seasonal variations were observed. From these facts it seems clear that we must not consider the dissolved organic matter as having been put more or less aside from the cylce, but as forming an integral part of it.

In order to check these facts Mr. DUURSMA set up an investigation nearer home on the seasonal variation of dissolved organic matter and the factors which may be responsible for it. To this purpose every fortnight water samples from the North Sea, taken near the lightvessel Texel, were investigated on their content of dissolved and suspended organic matter and of plankton. This series of observations will be continued in 1959.

Just as in previous years a number of water and bottom samples were investigated on behalf of "Rijkswaterstaat", department Hoorn. We must also mention that regularly water samples from the southern point of Texel were investigated in view of a possible future inlet of sea water for the new Zoological Station.

The research on the fouling of ships, carried out under the direction of Mr. DE WOLF at Den Helder by the Netherlands Organization for Applied Research, was brought over in June to a wooden laboratory, built to that end outside the Zoological Station. It has a private seawater circulation, which draws its water from one of the 35 m³ tanks of the Zoological Station. Besides an aquarium space, it contains a

separate air-conditioned room for the algae-nursery. The presence of these cultures is of great value to the Zoological Station so that the benefits of the neighbourship are mutual.

A close contact was kept with the Delta-Research Department of the Hydrobiological Institute, which started working in 1957 and continued in 1958, largely from Den Helder. There the chemist-hydrographer, Mr. HOEKMAN, found accommodation from March 1st, Mr. DEN HARTOG from July 15th, the analyst Miss J. DE JONGE from October 1st. The director, Mr. K. F. VAAS, who entered service on the 1st of July, came often to Den Helder, and the Max Weber made four trips to Zeeland on behalf of the work there. It is therefore clear that co-operation was close. The hydrographical work in that area was mentioned above. The work of Mr. DEN HARTOG consisted mostly of drawing up inventories for a starting-point. At the meeting of the Hydrobiological Society on January 30th he suggested a classification of the Delta-area on ecological-geographical grounds, as illustrated by a number of Amphipods and worms. For the sake of completeness it must be mentioned that Mr. BEEFTINK, also attached to the Delta-Research Department, is working on plant sociological subjects in Zeeland.

There were also regular contacts with the State Institute for Fishery Research at IJmuiden. Miss W. DE LIGNY of this institute spent a week at Den Helder in connection with her bloodgroup-determination of spring herring, which is still caught in very small numbers there. As always the contact with "Rijkswaterstaat, section Hoorn", was good. The schemes for extension of the Zoological Station met with sympathetic co-operation there.

The summer courses took up less time than in former years, since only 15 participants entered. Although this is to be regretted, it meant that our staff could spend more time on their own research. The participants were 5 students from Groningen University, 8 from Utrecht and 2 from Amsterdam. Mr. VAAS, the director of the Delta biological research, also joined the course. Separately, the Physiological Laboratory in Amsterdam gave a course for 24 of its own students, which was conducted by Mr. KRISTENSEN and Mr. PARMA. Further, the Free University, Amsterdam, also gave a course for its own students, in which 20 people took part.

The total number of man-days, spent in 1958 at the Zoological Station by research workers and course-participants, the Delta-people excluded, was about 1115. The average for the years 1947–58 is about 1120.

Visitors from abroad included Dr. W. Bergmann, Professor of Chemistry, Yale University, New Haven (Conn.), U.S.A., Dr. and Mrs Ralph Buchsbaum, Pittsburgh (Penns.), U.S.A., Dr. Walter A. Chipman, Beaufort (North Carolina), U.S.A., W. Hiatt, Honolulu, Hawaii, temporarily employed with the U.S. Office of Naval Research in London, Dr. Fergus O'Rourke, Professor of Zoology, Cork, Ireland, Dr. E. Schüz, Museum für Naturkunde, Stuttgart, Germany. The Zoological Station was also visited by 5 students of Prof. De Coninck, Ghent, Belgium, who came to study the method of collaboration between the Zoological Station and the Universities.

As considers the item Staff we have already mentioned that Mr. De Blok was taken over from the Netherlands Organization for Pure Research. He joined our staff on January 1st, 1958. Mr. G. De Boer was appointed as servant from Febr. 10th onward, and two new analysts entered our service: Mr. D. J. de Vries and Mr. J. W. Rommets, on March 1st and April 1st resp. Mr. De Vries took the place of Mr. T. de Vries, who entered service with the Research on fouling of the Organization for Applied Research. Starting December 1st Miss J. van der Vliet was appointed typist. To our regret Mr. Dijkstra was absent for 3 months owing to illness.

In connection with his work Mr. Duursma, with a grant of the Netherlands Organization for Pure Research, visited the German Hydrographic Institute in Hamburg and the Institut für Meereskunde in Kiel on 12–15 February and 10–12 July respectively, and Mr. Postma, who accompanied Mr. Duursma on his first trip, also visited Prof. Steemann Nielsen in Copenhagen on 23–28 March, in connection with his study of the CO_2-cycle. The trips of Mr. Dudok van Heel to Denmark were mentioned above. During his holidays Mr. Verwey spent a few days in the Laboratory of the Marine Biological Association at Plymouth, England.

In the past year especially Mr. Verwey devoted much time to the schemes for extension of the Zoological Station. After it had become more and more obvious that at Den Helder no proper site for a new building would be found our attention was concentrated on the southern point of the island of Texel. The "Rijkswaterstaat" obliged us by putting at our disposal valuable hydrographic data for that area, which in the course of 1958 were completed by data of our own. The distribution of salinity and temperature at the place in question begins to take shape. It must be mentioned that not only the Zoological Station itself, but especially Prof. Baerends as the President of the Society and Mr. Koster of the direction of the State Building Serv-

ice, devoted much time to discussions on these extension schemes.

Pending this question no alterations were made in the building itself, but we spent *f* 1000.— on upkeep of the roof, an amount of about *f* 1000.— on exterior painting and *f* 1500.— on painting the window-frames inside and the course-room. Moreover, in consultation with the Municipality, we put an end to the perilous condition of the electric mains in the building, which cost *f* 3000.— in 1958. It is a very great improvement that the old furniture could be replaced and that at the same time oil stoves took the place of the old coal stoves. This improvement took *f* 11 000. – so that the total expenditures on building and furniture amounted to *f* 17 500.—.

As the shortage of space made itself felt more and more strongly, after consultation with the Ministry we proceeded to buy a house near the Zoological Station which will serve as a students' lodge instead of the old one. It was bought for *f* 17 000. – and until the end of 1958 we spent *f* 20 000.— on building expenses. It may easily accommodate 30 people. The existing students' lodge "De Potvis" on the grounds of the Zoological Station will be used as a work shop. The acquisition of the house has been made possible by a Government grant of *f* 40000.— for 1959.

The budget for the library was enlarged from *f* 1100.— to *f* 2500.— starting with 1958. So this year for the first time we could buy a number of handbooks, which means a great asset to our work. Among them are part 1 and 2 of The Fishes by BROWN, HUTCHINSON's Treatise on Limnology, MOORE's Marine Ecology, DIETRICH's Allgemeine Meereskunde, a new Polish Atlas for the North Sea, a.o. Through intermediary of the Office of Naval Research our library obtained a number of valuable reports on the influence of radioactivity on marine organisms. The exchange proceeds normally. To make an end to the increasing lack of space, which is the consequence of these activities, the middle steel cases in the library were heightened, so that a space of 100 metres could be added. In this way it was possible to do away with the book cases in two of the working rooms and to bring all the periodicals together again. Unfortunately, the relief is only for one year, after that the problems will start all over again.

The item Instruments also went up starting with 1958, viz. from *f* 4000.— to *f* 6500.— with a temporary increase of *f* 8000.—. This made it possible to buy a.o. 8 Czech microscopes for the courses in the hope that it will be possible to avoid dragging private microscopes to Den Helder and back, at least for some of our visitors. Further we bought a Leitz Laborlux microscope, a Melag incubator and paraffine-stove, a vacuum pump, a muffle-furnace and a Ferrograph echo-sounder for the Max Weber. It must be mentioned that the Netherlands

Organization for Pure Research handed over the lunar and tidal apparatus of Mr. De Blok to the Netherlands Zoological Society.

In 1958 much use was made of the Max Weber, both in the Wadden area and in the province of Zeeland. The ship made 127 navigation days, which is a lot. In addition to the cost of upkeep of *f* 3500.— we had to spend an extra amount of about *f* 3000.— on a thorough revision of the clutch, which used to give trouble of late. The money was found on other items. Besides its echo-sounder the Max Weber got a wireless apparatus and butagas.

The income from study materials in 1958 amounted to *f* 10325.—. We bought animals for *f* 5525.— and spent *f* 2100.— on preserving liquids, wickerbottles and other materials for transport, so that the expenditures amounted to *f* 7625.—. This makes a balance of *f* 2700.—, which is *f* 600.— less than that of 1957. The Zoological Station is much obliged to the State Institute for Fishery Research, which took care of buying and preserving sharks at IJmuiden, since at Den Helder not enough sharks are supplied to meet all the demands. To the end of the year we bought a Ford delivery van, so that the transport to the Railways is now in our own hands. This saves a lot of extra-work and transport is quicker.

The total costs of exploitation, excluded the amounts granted by the Netherlands Organization for Pure Research, made *f* 220000.—in all. As compared to 1957 the amount has increased by about *f* 50000.—, to which another *f* 24000.— in single grants has to be added. This is all due to the goodwill of the Government regarding the extension schemes of the Zoological Station, of which a first stage was realized in 1958 in the form of amounts for extension of staff, acquisition of furniture and extension of the instrumentary.

Den Helder, 18th Febr. 1959 J. Verwey

COMPTES RENDUS DE LA SOCIÉTÉ NÉERLANDAISE DE ZOOLOGIE

JANVIER 1957 – DÉCEMBRE 1959

Séance du 12 janvier 1957 à Utrecht

1. M. DUIJM (Groningen). La communication acoustique chez les Orthoptères.

Un résumé de cette conférence n'a pas été remis au secrétaire.

2. J. J. M. L. CROMBACH[1] (Amsterdam). The effect of pre-service sexual excitation on sperm production and fertility of dairy bulls.

1. OBJECT OF THE RESEARCH. In several Artificial Insemination (A.I.) Centres different methods are used in the preparation of bulls for service. Usually the bull is restrained until protrusion occurs. In some centres bulls are restrained for a time even after protrusion, combined sometimes with one or more false mounts prior to service. These differences in methods are motivated primarily by intuition. Studies bearing on the efficacy of these different procedures are meagre and conflicting (BAKER 1954, BRANTON 1952, COLLINS 1951, HELLSTRÖM 1947, ISHII 1953, KERRUISH 1955, SOKOLOVA 1940). Most workers have found a favourable influence of preparation on sperm production. These investigations, however, have been too fragmentary to answer the following fundamental questions:

1. Is it possible to ameliorate the quality and to augment the quantity of the first ejaculate by preparing the bull?
2. If so, then what is the best kind of preparation?
3. Since it is usual to allow the bull to ejaculate twice successively, is it possible to improve the second ejaculate by preparation, while retaining any possible favourable effect achieved in the first ejaculate?
4. Will the effect of preparation eventually disappear because of adaptation of the bull to the changed circumstances?
5. Can this method, when applied in practice, improve the fertility of bull semen?

[1] Research Group on the Behaviour of Farm Animals T.N.O., Zoological Laboratory, Amsterdam. The author is much indebted to the Research Institute for Animal Husbandry T.N.O. at Utrecht for help in collecting the data included in this paper.

Theoretically it is plausible that a good preparation of the bull before service has a favourable effect on the ejaculate. It is well known that many instinctive activities require a certain running time, and psychosomatic factors may also play a role. It might, however, be possible that the bull may finally accommodate to the treatment, thus eliminating any advantage obtained by preparation. Only a long-term experiment can resolve these problems.

II. EXPERIMENTAL PROCEDURE. For want of space only the most striking test series are mentioned here:

1st Test series. No preparation versus *prolonged preparation*

One pair of identical twin bulls was used. One was allowed to serve twice, immediately after entering the serving-stable, without any preparation. The other was restrained for 10 minutes before the first ejaculation and for 5 minutes before the second. This procedure was continued for nine weeks during which time the roles played by the two bulls were interchanged once. The first ejaculate of the restrained bull contained, on an average, 2.9 times more motile spermatozoa than that of the non-restrained bull. Furthermore sperm wave-motion was superior.

2nd Test series. Slight preparation versus *thorough preparation*

One of a pair of bulls was allowed to serve twice immediately after protrusion. The other was restrained for 5 minutes, during which time he was urged to make one false mount, before the first ejaculate, and was restrained for 5 minutes before the second ejaculate. This test series was done with two pairs of identical twin bulls over a period of two months. Every fortnight the roles of the bulls in the experiment were exchanged. Sperm production by the restrained bulls in this series was found to be 3.2 times the amount obtained in their non-restrained brothers, i.e. superior to that of bulls in the first series.

3rd Test series. Slight preparation versus *the minimum effective preparation*

This series of tests was planned to determine whether it is possible te retain the favourable result of the second test series even though the 5 minutes restraining time is abandoned. Preparation of the test bull consisted of one false mount before every ejaculation. The control bull had to ejaculate twice immediately after protrusion. Three pairs of identical twin bulls were used. The roles of the bulls were alternated every fortnight. The first ejaculate of the bulls who made one false mount was substantially better than that of the controls, although it was generally not as good as that of the second test series.

4th Test series. Improvement of the *2nd ejaculate*

In this series 4 pairs of identical twin bulls received the maximum preparation before the first ejaculation. Before the second ejaculation one bull of each pair was prepared as before the first (restraining for 5 minutes and one false mount). The other was allowed to serve again without further preparation immediately after the first ejaculation. The second ejaculate of the prepared bulls contained on the average 1.4 to 1.9 times more motile spermatozoa than the second ejaculate of their unprepared brothers. The spermatozoa of prepared bulls also showed greater wave-motion. From these results is was concluded that preparation of bulls before the second ejaculation was also of great importance.

5th. Test series. Adaptation test

With five pairs of identical twin bulls an experiment was undertaken to investigate whether the advantageous effect of preparation would disappear in the long run, due to adaptation of the bulls to the preparatory treatment. The test bulls were used during 3 months without deviation in the experimental procedure. Before both ejaculations they were restrained for 5 minutes during which time they made one false mount. The control bulls were allowed to serve twice immediately after protrusion. The ejaculates of the restrained bulls contained significantly more motile spermatozoa than those of the non-restrained controls and the spermatozoa had greater wave-motion. A statistical evaluation of the results indicates no evidence for adaptation.

III. PRACTICAL APPLICATION. Because the semen of the twin bulls could not be used for insemination, tests comparing the fertility of prepared and non-prepared bulls are being carried out in cooperation with A.I. centres. Provisionally, preparation seems to aid fertility.

IV. CONCLUSION. There is sufficient evidence to indicate that preparation is a biologically important part of the breeding act of bulls. Preparation may be considered to be a naturally occurring phenomenon in herds of cattle. Because a cow does not remain standing to be covered until she has come fully into heat, the bull follows her and undergoes hours of preparation with many false mounts. This fact has been largely neglected by cattle breeders. It would, therefore, be of great importance to pay more attention to preparation than has hitherto been done.

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3. F. W. Reysenbach de Haan (Utrecht). Hearing in whales and dolphins.

A brief historical review of investigations on hearing in the Cetaceans shows that even the earliest writers have known that the auditory sense of this group is excellently developed. However, the manner in which transmission and perception of sounds are achieved, has remained obscure until the middle of the twentieth century.

Petrus Camper (1765) was the first to contribute materially to present-day knowledge of Cetacean hearing. Since then, no essential advances were made in this field until Fraser and Purves (1954) published their studies on the function of this sense organ. It must be mentioned that it was the development of anti-submarine defences, particularly during and after the second world war, which led to the discovery that Cetaceans are especially sensitive to supersonic sounds.

A survey of the differences in physical properties of sounds in water and air, respectively, shows that the aquatic environment must have a preponderant influence on the functional anatomy of the Cetacean ear.

Apart from hearing, no other sense organs can subserve the orientation of Cetaceans except at very close quarters, either because these organs are generally unsuitable for this function, at least under water, or because they are but poorly developed in Cetaceans. Therefore, auditory messages must be the main source of information in communication and in the perception of food or danger.

For physical acoustical reasons, an external auditory meatus must be void of function. The anatomical findings are in good agreement with this view.

The mechanism of the middle ear has been completely retained in Cataceans, but it is entirely rigid, especially as regards the attachment of malleus and stapes. Both the middle ear and the internal ear are surrounded almost completely by air, in casu foam. This must be regarded as an acoustical insulation, a necessary condition for sound localisation.

The extreme rigidity of the Cetacean middle ear has a parallel in other animals sensitive to supersonic sounds (Carnivores, Rodents, Microchiropterans). This can be explained from the fact that the middle ear is a mechanical transmission system. For, the more rigid such a mechanism, and the smaller its mass, the higher also are the frequencies it will pass.

The microscopical structure of the internal ear (organ of CORTI and surroundings) in Cetaceans again shows striking similarity to that of other animals with supersonic hearing.

Estimates of the maximum frequency a mammalian species can hear may be based on the structure of its middle and internal ear.

Finally, a survey is given of the central acoustical system of whales and dolphins. This is remarkably similar to that of the Microchiroptera. It can be stated that the entire brain has been built around the central acoustical system.

4. J. W. KUIPER (Groningen). L'unité fonctionnelle dans l'œil d' insecte.

Un résumé de cette conférence n'a pas été remis au secrétaire.

5. P. SEVENSTER (Leiden). The mechanism of a displacement activity.

If one of the various instincts, which an animal has at its disposal, is strongly activated, the others are usually inhibited. Quite commonly, however, we meet with situations, in which two instincts are activated at the same time. The behaviour then shown is clearly a mixture of activities, some of which belong to the one and some to the other instinct. Such behaviour is called ambivalent. It is particularly frequent in courtship and other social situations.

Ambivalent behaviour may be accompanied by activities, which cannot be ascribed to either of the activated instincts. At first sight this seems to suggest that yet another instinct is activated. Though this may be so in some cases, in others such an assumption seems not justified, because in the ambivalent situation those factors seem absent, which (from other situations) are known to be indispensable for the activity concerned to occur. From our knowledge of its usual occurrence, therefore, we would not expect it to appear in the ambivalent situation. An activity, occurring under these circumstances, is called a displacement activity. As its definition: 'A displacement activity is an activity belonging to the executive motor pattern of an instinct other than the instinct(s) activated' (TINBERGEN, 1952) does not imply an ambivalent situation, it may well be that displacement activities are not restricted to such situations, though so far most examples certainly are. TINBERGEN (1940) and KORTLANDT (1940), who independently discovered the phenomenon of displacement activities, also formulated a causal hypothesis to explain their occurrence. Various considerations led them to suppose that the nervous centre of an activity, when this occurs as a displacement activity, would be activated by impulses from the activated centre of an instinct, to which the activity does not belong. In the ambivalent situation one of the activated instincts or possibly both of them would thus activate a motor pattern, belonging to a third instinct.

As the hypothesis has been discussed repeatedly and has raised many difficulties, it seemed worth while to test it by an analysis of a typical displacement activity. For this purpose I chose the displacement "fanning" in the male three-spined stickleback *(Gasterosteus aculeatus L.)*, which has been cited in the literature as a good example of a displacement activity.

The movement of "fanning" is characterised by a special coordination of the fins and obviously serves the function of ventilating the eggs. Quantitatively the amount of fanning can be easily expressed in seconds of fanning per ½ hour. It occurs most frequently in the parental phase, i.e. when eggs are in the nest. Previous investigations (Van Iersel, 1953) have shown that the amount of fanning in this phase is increased by three factors: 1. the number of eggs, 2. their age, and 3. (internal) motivation. The effect of the first two factors, at least part of it, is elucidated by the fact that water, through which CO_2 has been passed, also increases the amount of fanning, when led through the nest. Fanning decreases, however, with an increase in sexual motivation. From these experiments we know of four activating factors (the CO_2-water included and one inhibiting, which influence the activity.

The same fanning activity, though in smaller amounts occurs in the sexual phase (i.e. before eggs are in the nest), as well as during courtship (irrespective of the phase!). This is unexpected; not only because, functionally, there seems to be no need of ventilation, but also because none of the activating factors mentioned above, seem to be present, while the inhibition by sexual motivation should be strong, as in this phase the animal is particularly ready to court a female intensively. On Tinbergen's definition, given above, it would seem that fanning in the sexual phase and during courtship can be designated as a displacement activity. It is then not surprising to find that the amount of fanning in the sexual phase increases with an increase in sexual motivation. For this immediately seems to point to the conclusion, that the fanning centre is activated by impulses from the sexual centre (*cf.* Tinbergen and Van Iersel, 1947). Nevertheless this is definitely in contrast with the inhibitory relation, found in the parental phase. This difficulty could only be overcome by the additional hypothesis that the transition from the sexual into the parental phase coincides with a change in the relation between the two centres. Experiments, however, indicate another way out of this difficulty and support an entirely different hypothesis.

First of all it appeared that, though fanning in the sexual phase is correlated with sexual motivation, it is also always connected with ambivalent behaviour, the two instincts activated being sex and aggression. This is especially clear, when it occurs during courtship, which in this

species is a mixture of sexual and aggressive activities. The two groups of activities may occur in different ratios, so that by counting them under standard conditions it is possible to determine the state of balance of the two centres involved. It is with this balance, and therefore probably with some sort of conflict between the centres, that the amount of displacement fanning is most clearly correlated.

Secondly it appeared that displacement fanning could be "facilitated" by all the activating factors, which had been found to be effective in the parental phase. If *e.g.* CO_2-water was added to the nest during courtship, there was more fanning than in the control periods with a comparable balance of sex and aggression. Number and age of eggs and (internal) motivation would also increase the amount of fanning in courtship. With equal doses of these factors, however, this facilitating effect was less the stronger the sexual motivation during the courtship period concerned, so that when the balance was shifted completely in favour of the sex, no facilitating effect could be detected. This could be plausibly explained by the assumption, that also in displacement fanning the inhibition by the sexual centre is at work. We must then conclude that, given a certain balance of sex and aggression, the amount of fanning is controlled by the same factors as in the parental phase. It seems likely, therefore, that the fanning centre is activated in the "normal" way, but how should we then account for the correlation with the state of balance of sex and aggression ? Within the scope of this paper it is impossible to elaborate on this point, but so much can be said that we reach a fairly coherent picture of the displacement mechanism, if we assume that the role of this balance, or perhaps better, the conflict is not to exert an activating influence on the fanning centre, but to the contrary to remove the inhibition on it, caused by the sexual centre. If we further imagine some activating factors, however weak, to be always present, even in the sexual phase, it can be understood that removal of this normally strong inhibition will lead to the occurrence of the fanning activity. The activating factors, so to say, are only allowed expression, when a conflict between sex and aggression arises. It is suggested that the nature of such a conflict might be that the sexual centre itself becomes for a moment inhibited by the aggression gaining dominance. This would naturally make it impossible for the sexual centre to maintain its inhibition on the fanning centre.

The frequency of such a conflict, however, would depend on the state of balance between sex and aggression, which would explain the correlation of this balance with the amount of fanning. The hypothesis can be conveniently called the disinhibition hypothesis.

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Séance du 16 février 1957 à Amsterdam

H. Kleerekoper (Hamilton, Canada). L'ouïe des poissons. Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 23 mars 1957 à Leiden

1. H. Boschma (Leiden). Remarks on specificity of parasites.

In some groups of parasites the species are restricted to a single host species or to a group of species of close taxonomic relationship; perhaps the Epicaridea form the most striking example of such a connexion between parasite and host. Though in Rhizocephala many instances are known of parasites that occur exclusively on one species of host, and several hosts appear to be infested by only one species of Rhizocephalan parasite, this is by no means a fixed rule. Some crabs from the Indo-pacific region may bear parasites belonging to up to six different species of Sacculinidae, each of which in its turn may infest other hosts. The crab *Thalamita admete* (Herbst) forms an example of a species with six Sacculinid parasites, viz., two species of the genus *Sacculina*, two of *Heterosaccus*, and two of *Loxothylacus*. In New Guinea and adjacent islands the crab was found to be infested with three of these species of parasites, each apparently occurring in a restricted locality within the region.

2. K. H. Voous (Amsterdam). Limits of the species concept.

The species concept implies not only morphological, but also reproductive, ethological and ecological barriers. These barriers can be crossed in a number of ways, which are often surprising. Instances of the crossing of the species-barrier have been given, which are mainly taken from ornithological examples. The theoretical consequences of the species concept have been discussed.

3. H. Engel (Amsterdam). *Tullia*, a subgenus of *Aplysia* with a sucking disk at the posterior end of the foot.

In *Tullia*, a subgenus of *Aplysia*, the ability present in all *Aplysias* to adhere to some substrate with the posterior part of the foot, results in the development of a sucking disk. It appears that only those specimens which were actually using their posterior pedal glands and were

clinging to the rock with the hind part of the foot at the time of preservation, retain the sucker as a round disk distinctly separated from the rest of the foot when preserved.

In life the anterior part of the foot may also function as a sucker (though it is not preserved as such), allowing the animal to loop like a caterpillar.

A paper written on the subject together with Miss Dr. N. B. EALES (Reading) will be published in "Beaufortia", 1957.

4. D. A. HOOIJER (Leiden). Race and sex in the silvered leaf monkey *(Trachypithecus cristatus)*.

Various subspecies of *Trachypithecus cristatus* (RAFFLES) have been distinguished: *T.c.sondaicus* (ROBINSON et KLOSS) from Western Java, almost totally black; *T.c.pyrrhus* (HORSFIELD) from Central and Eastern Java, with grey-tipped hairs; *T.c. kohlbruggei* (SODY) from Bali, similar in colour to the last; *T.c.cristatus* (RAFFLES) from Sumatra and Borneo, with greyer hair tips and paler ground colour; and *T.c.ultimus* (ELLIOT), likewise from Sumatra and Borneo, which is a still paler form. Erythristic mutants are known to occur both in Eastern Java and in Northern Borneo; most of these individuals are males.

A craniometrical study of all of these races shows that the female skull is smaller than the male, with a proportionally wider brain case and greater height, etc. There prove to be racial differences in the size of the male skulls relative to that of the females, as follows below.

The male skull of *pyrrhus* is larger than that of *sondaicus*, and the difference in size is statistically significant. There is no difference in size between the female skulls of the two Java races, however. Hence, the secondary sexual difference in skull size is greater in *pyrrhus* than it is in *sondaicus*.

Both the male and the female skulls of *kohlbruggei* do not differ from those of *sondaicus;* there are no craniometrical differences between the Bali and the Western Java races, which are quite distinct in fur colours.

The male skulls of *cristatus* and *ultimus* are as large as those of *sondaicus*, but the female skulls are smaller than those of *sondaicus*, and the difference in size again has statistical significance. Secondary sexual differences in skull size, therefore, are more marked in *cristatus* and *ultimus* than they are in *sondaicus*.

Very little material is available from Billiton; the male skull (Leiden Museum) is smaller than that of *sondaicus*, *cristatus*, or *ultimus*, but the female skull from Billiton (U.S. National Museum, Washington) is as large as that of *cristatus* and *ultimus*. Therefore, in the Billiton form the secondary sexual difference in skull size appears to be less marked than that in the Sumatra and Borneo races. The form from Billiton (most probably entitled to a distinct subspecific name) appears to differ from

cristatus and *ultimus* in the same point as that in which *sondaicus* and *kohlbruggei* differ from *pyrrhus:* the male skull only is smaller, the female skulls being equal in size.

Consequently, we are now able to distinguish four race groups of *Trachipithecus cristatus*, each of which differs from that immediately preceding it in the size of the skull of one sex only:

	I. *pyrrhus*
♂ skull smaller:	II. *sondaicus*, *kohlbruggei*
♀ skull smaller:	III. *cristatus*, *ultimus*
♂ skull smaller:	IV. (Billiton)

The above arrangement of the races of *Trachypithecus cristatus* according to decreasing skull size deviates somewhat from that based on external characters; *sondaicus* is the darkest, and *ultimus* is the palest form of all. The female from Billiton is indistinguishable externally from *cristatus*. Nevertheless, the results of this craniometrical study underline the racial distinctions thus far mainly based on fur colours.

5. J. H. Stock (Amsterdam). Geographic speciation in Pycnogonida.

Voir: Beaufortia, **6**, 1957.

Séance du 11 mai 1957 à Wageningen

1. K. van Asperen (Utrecht). Sur la spécificité des cholinesterases chez les mammiféres et les insectes.

Voir: Meded. Landb. hogesch. Opz. stations Gent, **22,** 1957, 447–456; Nature, **181,** 1958, 355–356; Van Asperen en Dekhuyzen, Biochim. Biophys. Acta, 1958.

2. F. J. Oppenoorth (Utrecht). La résistance contre les insecticides.

Voir: Nature **181,** 1956, 425–426.

3. N. Prop (Groningen). The lateral ocelli of the larvae of *Diprion pini* L. (Hymenoptera).[1]

A very remarkable property of the larvae of *Diprion pini* L. is their reacting to moving objects. The response can be for example a rapid jerking up- and downwards of the anterior part of the body or a stretching of this part in the direction of the moving object. This susceptibility to visual stimuli being an uncommon phenomenon among larvae, it seemed worth analyzing the optical power of the *Diprion* ocelli.

In the analysis larvae of 22 mm length were used. The applied technique was for the greater part based on Homann (1924).

Anatomically, the two lateral ocelli of *pini* are characterized by a biconvex lens, a layer of corneagen cells and a cup-shaped retina, composed of sensory cells aggregated in groups of four.

The image formed by the lens proved to be clear and sharp. The focal length, as the next point in the optical analysis, has been deter-

[1] A more extensive account of the results will be published elsewhere.

mined experimentally, following HOMANN's method (slightly modified), and with a lens formula.

Four experimental determinations, based on measurements of the distance between the formed image and the lens top, gave focal lengths of 225, 250, 251 and 264 μ, with an average of 248 μ. By means of a second procedure, involving determinations of the curvature of the outer lens surface and the distance between its centre of curvature and the focal plane, a focus value of 259 μ was obtained. The lens formula gave a value of 246 μ.

The agreement of the results of the three methods is very satisfactory. It must be stressed, however, that the applied procedures are not so exact that we might have expected such a narrow variance in the results.

We must here remark that *pini*-larvae can respond when something is moving at a distance of *e.g.* two centimeters. As compared with the focal length, the distances of the objects to which the larvae react are so large also that their images will always lie in the focal plane.

The distance from the lens top to the base of the rhabdoms, determined by means of paraffin sections of the ocelli, was 217 μ, and to the outer limit of the rhabdoms 172 μ. The influence of shrinking being unknown, the values of 217 μ and 172 μ are maintained in a comparison of the location of the focal plane in the lateral ocelli of *pini* and other, anatomically comparable, ocelli.

In *pini* the focal length (taken as 248 μ) is 1.1 $\times$ the distance between the lens top and the bases of the rhabdoms, or 1.4 $\times$ the distance lens top — outer limit rhabdoms. In the literature we found the following comparable facts: the focal length of the dorsal ocelli in *Eristalis* is 2.6 $\times$ the distance lens top — outer rhabdom limit (HOMANN 1924); in the dorsal ocelli of *Locusta* the focal length is 2.1 $\times$ the distance lens top — rhabdom bases (CORNWELL, 1955); in the lateral ocelli of the larva of *Cincindela* the focal distance is 1.4 $\times$ the distance lens top — outer rhabdom limit (FRIEDRICHS, 1931).

From the above facts the interesting conclusion can be drawn that the dorsal ocelli in the two adult insects are, from a physical point of view, inferior to the lateral ocelli of *pini* and the *Cicindela*-larva: the location of the focal plane is evidently better in the latter. We can interpret the physical inferiority of the dorsal ocelli in the light of our knowledge about their physiological significance: they cannot induce responses, they can only influence responses induced by the compound eyes. At first sight, it may seem surprising that the ocelli of the *pini*-larva, which is phytophagous, are of the same relatively high physical quality as those of the *Cicindela*-larva, which attacks small prey passing its hole in the sand. However, behavioural observations on *pini* indicate

that its reactions to moving objects afford protection, to some extent, against parasites.

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HOMANN, 1924: Z. vergl. Phys., 541–578.

4. J. RINGELBERG (Amsterdam), aussi de la part de J. H. KUCHLEIN. Quelques remarques sur la distribution des insectes hivernant dans les fosses de marne du Limbourg du sud.

Voir: J. H. KUCHLEIN en J. RINGELBERG, Natuurhist. Maandblad, **45**, 1956, 125–131.

5. G. BECHT (Utrecht). Recherches sur le système neuromusculaire de la blatte.

Voir: Diss. Amsterdam 1959.

Séance du 14 octobre 1957 à Putten

1. H. KLOMP (Wageningen). The present state of the study of bird migration. A review.

The data and views put forward in this lecture were mainly derived from the following publications:

FARNER, D. S., 1955: in Recent Studies in Avian Biology. Univ. Ill. Press, Urbana.
KRAMER, G., 1952: Ibis, **94**, 265–285.
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MATTHEWS, G. V. T., 1955: Bird Navigation, Cambridge Univ. Press.
VERWEY, J., 1949: Bijdragen Dierk., **28**, 477–504.

2. A. C. PERDECK (Leiden). L'analyse de la migration de l'étourneau au moyen de déplacements.

Voir: Ardea, **46**, 1958, 1–37.

3. J. H. MOOK, (Kampen) aussi de la part de J. ROOTH et J. J. ZIJLSTRA. Observations sur la migration des oiseaux dans le nord de la Veluwe.

Voir: Limosa, **30**, 1957, 76–83.

4. F. CREUTZBERG (Den Helder). Use of tidal streams by migrating elvers (*Anguilla vulgaris* Turt.).

Voir: Nature, **181**, 1958, 857–858.

5. B. J. LEMPKE (Amsterdam). Problems of migration in the Lepidoptera.

Migration is a rather widely dispersed phenomenon in Insects. It is known to occur among Hymenoptera, Odonata, Coleoptera (especially Lady-birds, species of *Coccinella*), Diptera (some species of Hover-flies, Syrphidae), but especially among Lepidoptera. In this order some hundreds of species are known to migrate regularly, especially

in tropical countries. But also some of our best known species, such as the Red Admiral (*Vanessa atalanta L.*) and the Silver γ Moth (*Autographa gamma* L.) belong to them. Among our so-called Macrolepidoptera about 10% are known to migrate. With the Microlepidoptera the percentage is much lower.

A displacement of insects must fulfill some conditions to be considered a migration. First of all the animals must be able to exercise influence on the direction of flight. Insects carried by the wind (*e.g.* plant lice, young caterpillars with long hairs) are not migrating. Moreover the flight must take place more or less in a certain direction. A displacement at random, such as we often see with the Brimstone Butterfly (*Gonepteryx rhamni* L.) is no migration.

In older literature there are many published observations relating to migrating Lepidoptera. But the great interest for the problem arose after the publication of the book of C. B. Williams, The migration of Butterflies (1930). Soon after this English entomologists started to collect data about the migrating Lepidoptera occurring in their country and in the course of about 25 years an enormous quantity of observations has been brought together. In our own country I started with the same work in 1940, so that we now dispose of the data collected in 18 succeeding years, which have revealed many interesting facts about the species concerned. Other European countries in which the migration of Lepidoptera was more or less seriously studied, are Germany, Austria, France and Spain.

There is a striking resemblance between the migration in birds and in Lepidoptera. In both the principal direction of flight in the northern hemisphere in spring is from south to north, and in autumn from north to south. An important exception to this rule are the Cabbage Whites (*Pieris brassicae* L. and *P. rapae* L.), which arrive here from territories north-east of our country, most probably from the bords of the Baltic.

Though there can be no doubt that migration is a hereditary quality, a conclusive explanation has not yet been given. For the different views on the subject I must refer to the literature concerned.

A very interesting question is the problem of the return flights. It is best known of the North American Monarch (*Danaus plexippus* L.), but the recent abundant observations have clearly shown that at least in several of our migrating Lepidoptera return flights in autumn are no exception. In our own country *Vanessa atalanta* has repeatedly been observed to fly back in small numbers in autumn.

If migration has always a real advantage for the species, is doubtful. Many cases are known of swarms which flew seaward in a direction that they could never reach land again, and also on our shores thousands of drowned specimens have sometimes been observed. A quite

different case is that some species migrate to countries, where the conditions are so unfavourable for them, that the adults resulting from the eggs laid by the migrants are partly or completely sterile, at least the females. This is for instance the case with the Death head Hawk moths (*Acherontia atropos* L.) and the Convolvulus Hawk moths (*Herse convolvuli* L.), which are born in our country. But it is very probably also the case with an important number of the females of *Autographa gamma* L. Breeding experiments in Sweden have shown that this sterility is caused by our cool summer.

For an ampler discussion of the different problems raised in this article I may refer to my booklet on De Nederlandse Trekvlinders, published in 1957 by W. J. Thieme & Cie., Zutphen, at the end of which also an extensive list of the literature on the subject is given.

Séance du 16 novembre 1957 à Amsterdam

1. C. P. Raven (Utrecht), aussi de la part de Mlle. F. C. M. Escher, W. M. Herrebout et J. A. Leussink.

Voir: J. Embryol. Exp. Morph., **6,** 28, 1958.

2. W. J. van Dongen (Utrecht). Sur le mécanisme de la spermiation chez la grenouille (*Rana temporaria*).

Un résumé de cette conférence n'a pas été remis au secrétaire.

3. A. Kortlandt (Amsterdam). Behaviour research on the evolution of apes and man.

1. From a comparitive zoological point of view, the great apes are, in their behaviour, strikingly related to man, and very different from the monkeys (homologous expressions; formal course of ontogenetic development, etc.). Moreover, the apes have some sort of 'culture' or 'protoculture' which is socially transmitted from one generation to another (vegetarianism, nest-building, etc.). And, finally, their cultural potentials are in several respects almost human —far in excess of their natural requirements (Viki experiment). This being so why have the apes not developed a more human way of life, and how could they, in the course of evolution, ever develop the cultural over-capacity which they normally do not use or need?

2. From an ecological point of view, man is essentially a weapon-using, cooperative hunter, specialized for large prey, whereas the apes are mainly herbivorous and in some degree insectivorous. This difference is reflected in quite different types of 'cosmological' (Umwelt) organization (a world of movables in man, a world of immovables in the apes). And further, man is a ridiculous species, in contrast with all apes and monkeys, but in line with all carnivores. In a certain sense, the most human animal is the wolf (convergent evolution: cooperative hunting technique, social organization, monogamy, super ego, aspects

of domestication, etc.). Man and ape represent divergent evolutionary lines.

3. Curiously, however, there are some inconsistencies in the picture of the apes just given: alimentary physiology; ecological surplus of intelligence; spontaneous use of objects as throwing, stabbing and clubbing weapons in play; false hunting in play; an experiment by M. Kooy, etc. All this evidence suggests what might be called 'post-protohominid behaviour" in the apes: man has not so much descended from an ape-like ancestor, but rather the apes have descended from a more man-like, predominantly ground-living species, partly hunting by means of weapons, living in bands but not hunting cooperatively, and inhabiting a more or less open savannah or park landscape. It would seem that the proceeding evolution of the hominid line has gradually expelled the anthropoid side-branch to the tropical forests, where they secondarily developed brachiating and exclusive vegetarianism, and where the human features in their behaviour subsequently degenerated. Thus they are by no means "infra-human".

4. This tentative hypothesis finds corroboration in evidence derived from species which are, from an ecological point of view, more protohominid than the recent apes: defensive throwing of objects in baboons and some macaques versus defensive dropping of objects in arboreal monkeys; predatory behaviour in baboons; sociological aspects. Moreover, the hypothesis is consistent with fossil evidence and other considerations (*Proconsul;* new finds of *Gigantopithecus;* analogies in cultural anthropology). More research should be done in the great apes with regard to (1) the motor patterns involved in throwing, (2) the potentialities of living in the paleolithic way under experimental conditions, (3) the ecological backgrounds under natural conditions.

4. J. A. Eygenraam (Arnhem). Recherches sur le gibier de chasse dans les Pays Bas, en particulier les oiseaux aquatiques.

Un résumé de cette conférence n'a pas été remis au secrétaire.

5. J. Luitjes (Arnhem). Sur la présence de *Diprion* et *Cephalcia*.

Un résumé de cette conférence n'a pas été remis au secrétaire.

6. W. Vervoort (Leiden). On the pituitary cytology of *Lebistes reticulatus* (Peters).

Voir: Proc. Kon. Ned. Akad. Wet. Amsterdam, ser. C, **60,** no. 4, 1957.

Séance du 14 décembre 1957 à Utrecht

1. G. P. Baerends (Groningen), aussi de la part de K. H. Posthuma et Tj. Joustra. Die Reaktion der brütenden Silbermöwe auf die Temperatur des Eies.

Wenn man Eier aus dem Gelege einer Silbermöwe wegnimmt oder durch abnorm gestaltete Eiattrappen ersetzt, steht die brütende Möwe,

wenn sie zum Nest zurückgekehrt ist, wieder auf, setzt sich anders hin, ordnet die Eier um, oder fängt an wie im Übersprung zu bauen oder sich zu putzen. Um ruhig weiterbrüten zu können, muß der Vogel offenbar bestimmte Rückmeldungen vom Gelege empfangen.

Man wird fragen, ob auch die richtige Eitemperatur zu diesen Rückmeldungen gehört und ob der Vogel auf von normalen abweichenden Temperaturen deutlich anspricht. Ist dies der Fall, so könnte man sich vorstellen, daß bestimmte Rückmeldungen Verhaltensweisen auslösen, die zum Wiederherstellen der richtigen Bruttemperatur dienen und daß diese Reize den Bruttrieb und vielleicht auch andere Triebe quantitativ beeinflussen. Außerdem liegt es nahe zu fragen, ob die Reaktion auf Rückmeldungen vom Gelege von der Höhe des Bruttriebes abhängt.

In unseren Versuchen ersetzten wir das normale Gelege durch natürlich bemalte Eier aus Kupfer, die elektrisch beheizt oder mit durchströmendem Wasser gekühlt werden konnten. Die Temperatur dieser künstlichen Eier und auch die von normalen Kontrolleiern wurde im Beobachtungszelt, einige Meter vom Nest entfernt, mit Widerstandsthermometern abgelesen. Von hier aus konnte man die Temperatur der beheizten Eier mit einem Widerstand, die der gekühlten mit einem Quetschhahn regeln und konstant halten. Die Vergleichstemperatur war 37°C; wir erwärmten in verschiedenen Versuchsreihen auf 45° und 50° oder kühlten auf 25 und 17° ab. Jeder Versuch dauerte 45 Minuten, vorher und nachher beobachteten wir ebenso lange bei normaler Temperatur. Das Verhalten der Vögel wurde fortwährend beobachtet und protokolliert. Es zeigt sich zuerst, daß es bestimmte Verhaltensweisen gibt, die der Regulierung der Eitemperatur dienen. Wird das Gelege zu heiß, so sträuben die Tiere das Gefieder, bei Abkühlung plustern sie sich auf. Erwärmung löst Hächeln und Aufstehen, Abkühlung dagegen Schaukeln und Kältezittern aus. Frequenz und Intensität dieser Handlungen sind umso höher, je mehr die Temperatur von der normalen abweicht.

Besonders wenn die Abweichungen größer werden und zwar beim Erwärmen viel früher als beim Abkühlen, nehmen das Aufstehen und sich anders Hinsetzen sowie das Umordnen der Eier zu.

Dies zeigt, daß die Brutstimmung verhältnismäßig abnimmt, während der Drang, das Nest zu verlassen, gleichzeitig immer größer wird. Zugleich beginnen Übersprungbewegungen, und zwar Bauen sowohl beim Erwärmen wie beim Abkühlen und Putzen besonders beim Erwärmen und bei starker Abkühlung. Das Putzen scheint besonders häufig zu sein, wenn das Tier im Konflikt zwischen beiden sich gegenseitig hemmenden Tendenzen mehr zum Verlassen des Nestes neigt. Bauen herrscht vor, wenn die Brutstimmung relativ stärker ist.

Das Verhalten hängt nicht allein von der Eitemperatur, sondern auch von der Außentemperatur ab. Dies erschwert die Versuche im Freien sehr.

Beobachtungen bei verschiedenen Wetterverhältnissen lassen vermuten, daß die Vögel am stärksten auf die Rückmeldungen vom Gelege ansprechen, wenn die Brutstimmung nicht sehr hoch (wie z.B. bei Regenwetter) oder sehr niedrig ist (wie z.B. bei warmem Wetter).

Die Eitemperaturen sind besonders konstant, wenn Messungen am Verhalten eine ziemlich hohe Tendenz zum Brüten aufweisen; sie fallen sofort wenn die Brutstimmung heruntergeht, zum Beispiel weil in der Kolonie alarmiert wird.

2. P. D. Nieuwkoop (Utrecht). The neural competence of the ectoderm in Amphibians.

Following Holtfreter's investigations (1938) concerning the competence of the ectoderm, recently Gallera (1952) and Chuang (1955) have published the results of extensive investigations on the same subject. These three authors worked each with a different Urodele species, and each with a different method of analysis. However, the more diverse the ways of approaching a problem, the deeper one may eventually penetrate into it. This consideration has led to a renewed investigation into the changes occurring in the neural competence of the ectoderm, an investigation carried out with the help of a different method of analysis, viz. the fold implantation technique (Nieuwkoop 1952). In order to ensure the possibility of a later quantitative analysis, the investigation was carried out on eggs of the Axolotl *(Amblystoma mexicanum)*, large numbers of which can be obtained from one female. The operations are, however, so time-consuming that one batch of eggs is not sufficient to cover the entire developmental period under investigation. The material, therefore, consists of four series of experiments. Three of these show a close parallelism, whereas the fourth series shows a somewhat different behaviour.

The folds, consisting of ectoderm which had never been in contact with mesoderm, were always implanted into the anterior to middle region of the presumptive rhombencephalon of host neurulae (stage 14–15) from the same batch. This was done in order to keep the intensity of the inductive actions as constant as possible. From the investigations of Nieuwkoop and others (1952–'55) and of Sala (1955) it is known that in this region of the presumptive rhombencephalon two different inductive actions are exerted, viz. 1) a strong activating influence, which incites the ectoderm to neural development (after which the activated material develops further in a prosencephalic direction), and 2) a rather weak transforming action, which acts on the activated

material and changes development towards the formation of more caudal structures of the central nervous system. The combined action of both inductive influences on an implanted fold of fully competent ectoderm, into which principle (1) extends further than principle (2) leads to the formation of a secondary nervous system, consisting, in proximo-distal sequence, of rhombencephalon, mesencephalon, diencephalon and telencephalon, with their respective accessory structures.

If we consider the results of the present experiments from a purely qualitative point of view, we find that in the series no. I, III and IV all the brain sections mentioned above are present if the fold material is taken from the stages 7 till 11¼. In older fold ectoderm all neural structures fail to appear practically at the same time, except for some rhombencephalic structures which still appear until stage 11½. After this stage only some rhombencephalic neural crest structures develop. Contrary to this, in series no. II, which starts with fold ectoderm of stage 11½, the rhombencephalic structures appear until and even beyond stage 12½.

First of all we may state that the proximo-distal extension of the activating, respectively transforming principles into an ectodermal fold is a measure for the intensity of these actions and for the reactivity of the fold ectoderm to these influences. The inductive actions being constant, the fact that the distally situated prosencephalic brain sections fail to appear earlier than the proximally situated mes-and rhombencephalic (i.e. transformed) structures suggests that the reactivity of the ectoderm to activation decreases more rapidly than does the reactivity of activated ectoderm to transforming influences.

The difference in reaction between the series no. I, III and IV on the one hand, and series no. II on the other is essentially quantitative. It appears that in series no. II the decrease in competence proceeds at a markedly slower rate, so that the above mentioned phenomenon becomes more evident.

The very early decrease of the reactivity of the ectoderm to activation in the Axolotl shows a very interesting parallelism with the minimal time of action needed for activation of the ectoderm in this species. Miss Johnen (1956) has shown that for activation of fully competent Axolotl ectoderm the minimal time of contact with the archenteron roof is only 5–15 minutes (whereas a period of contact of at least 10 hours is needed in order to produce transformative effects). It is therefore evident that the activation is completed as soon as the archenteron roof has come to occupy its definite position underneath the ectoderm, which happens around stage 11. Consequently, the competence of the ectoderm for activation apparently disappears soon after that stage.

Gallera's results show that in *Triturus alpestris* the competence

of the ectoderm for neuralization remains present for a considerably longer period. Now, again according to the work of Miss JOHNEN (1956) *Triturus* ectoderm requires a much longer period of contact with the archenteron roof (at least 4 hours) in order to become neuralized. Similar relationships between neuralization time and duration of competence may exist in *Cynops orientalis* (CHUANG, 1955). Apparently there are pronounced interspecific differences.

A careful analysis of the neural structures formed in the series no. I, III and IV brings out another feature. From stage 10¾ onwards the differentiation of the prosencephalic structures in the folds becomes modified. Their external surface, which is in contact with the surrounding mesenchyme, begins to show a frayed instead of a smooth appearance. Individual cells get loose and are apparently changed into neural crest elements. In the folds taken from stage 11 this process of disaggregation already goes so far that the various regions of the prosencephalon are hardly recognizable any more. In fold material from stage 11½, in which all the prosencephalic structures fail to appear, all at once ear vesicles are found, which indicates that now also the neural crest structures have a rhombencephalic character. The observed disaggregation marks the beginning of the decrease of the competence of the ectoderm for neuralization. From investigations of SALA (1956) it is known that neural crest elements arise from neural structures under the influence of weak transforming actions. The disaggregation described above may then be taken as an indication of a decrease of the reactivity of the ectoderm to activating stimuli, combined with an undiminished or perhaps even increased reactivity of the activated cell material to transforming influences. Under these circumstances the transforming principle may manifest itself also in the more distal portions of the activated material.

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3. J. BETEL (Amsterdam), aussi de la part de A. PUNT, Mlle. W. PARSER et H. VAN GEEST. CO_2-transport in insect haemolymph.

In connection with investigations on the periodical release of carbon dioxide (CO_2) in several insects and in diapausing pupae of Lepidop-

tera (PUNT, 1950; BUCK, KEISTER and SPECHT, 1953; BUCK and KEISTER, 1955), we thought it worthwhile to determine the CO_2-equilibration curve of insect haemolymph. Many data on CO_2 content of the haemolymph are given in the literature, but on the CO_2 capacity of the haemolymph our information is scanty (LEVENBOOK, 1950; PUNT, 1957).

We therefore estimated the CO_2 content of haemolymph after equilibration with different tensions of CO_2

The haemolymph was obtained from *Hydrophilus*-beetles by decapitating the insect and collecting the haemolymph on a watch-glass. The sample was put in a 50 ml saturation flask and equilibrated with a gasmixture of atmospheric pressure containing a certain percentage of CO_2, by rotating the flask for one hour in a waterbath at 20° C. After equilibration a haemolymph sample was analysed with the Haldane bloodgas analysing apparatus. The gasmixture was analysed too.

TABLE I

The CO_2 capacity of haemolymph of *Hydrophilus caraboides* (20° C)

p CO_2 (mm Hg)	vol % CO_2	p CO_2 (mm Hg)	vol % CO_2	p CO_2 (mm Hg)	vol % CO_2
2	60.2	63	93.7	141	97.9
16	49.4	69	86.0	144	99.0
25	54.5	81	76.6	146	94.6
28	43.7	110	91.5	158	96.6
36	55.3	121	101	165	87.0
43	70.1	138	118	188	142
48	59.7				

The results are given in Table 1. The data have been reduced to 0° C. and 760 mm Hg, so that the amount of CO_2-total could be plotted against p CO_2. The tangent of the regressionline, thus found, is 0.4. The CO_2 capacity of the haemolymph therefore is 4 times that of pure water. The correlation coefficient found was 0.8; according to Student's t-test this is very significant.

From our experimental data it is clear that the CO_2 capacity of the haemolymph of *Hydrophilus caraboides* far exceeds that of pure water. It is therefore quite probable that the gaseous CO_2 produced by the tissues is first stored in the haemolymph before it is eliminated from the body by diffusion. How this storing occurs is not at all clear. There is evidence that carbonic anhydrase is not present in insect haemolymph and that carbaminoformation does not occur (LEVENBOOK, 1950; VAN GOOR, 1948).

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4. J. Lever (Amsterdam). Secretory phenomena in and near the central nervous system of some Pulmonates.

Voir: Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **C 60,** 510–522, 1957; **C 61,** 235–242, 1958; **C 62,** 139–144, 1959.

5. K. H. Voous (Amsterdam). Impressions de voyage sur les recherches zoologiques en Afrique du sud.

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 25 janvier 1958 à Leiden

1. K. H. Postuma (IJmuiden). Vertical migration in the herring.

The diurnal vertical migration in the herring is generally related to the changes in light conditions in the course of day and night. The behaviour of staying near the bottom during the daylight hours and of rising to the surface at night, which is generally observed in the North Sea, is usually explained in terms of a supposed low light-optimum in the herring.

Balls (1950) used his theory of light avoidance to explain this behaviour. He observed that herring schools in the Norwegian-deep did not descend to the bottom at daytime but stayed at a depth of some 75 fathoms. Light conditions at a depth of 75 fathoms were at daytime presumably such as are preferred by the herring.

Richardson (1952) observed that a herring school sojourning in midwater at daytime rose when the amount of light diminished due to a decreasing transparency of the water. Richardson observed also that a herring school descended at night to a certain level, though not as far as the seabottom, when a strong light was directed at the sea surface.

These observations of Balls and Richardson indicate that the factor light may indeed play an important part in the herring's diurnal vertical migration.

If the existence of a low light-optimum is the only factor governing the diurnal vertical migration one would expect that the herring-shoals rise to the surface layer every night. This, however, is not the case. Quite often one can observe that the herring schools do not reach the surface layers, but stop their ascent at a certain level below the surface.

Schubert (1952) connected this phenomenon with the existence of a thermocline, which should work as a barrier and prevent the herring to rise to the upper water layers.

Richardson (1952), who observed a case in which the ascending herring did pass the thermocline and stayed in the water layer just above the steepest temperature gradient, stated: "Any suggestion that the level at which the shoals are to be found at night was due to the presence of a thermocline, can be abandoned."

In the thermoclines of Schubert the temperature differed 6° C over 3 meters, and 2° C over 5 meters, in those observed by Richardson 1.65° C over 5 meters.

Obviously the two authors mentioned disagree about the possibility that the thermocline is a factor limiting the nightly ascent of the herring.

It seemed worthwhile to make a further study of the influence of the temperature factor on the diurnal vertical migration in the herring, to obtain a clearer understanding of the factors involved.

Method. The vertical migration in the herring was studied by means of echo sounder observations. Simultaneously the light and temperature conditions in the various water layers were determined with selenium photocells and a bathythermograph.

The observations were made during cruises of the research vessels "Antonie van Leeuwenhoek" and "Willem Beukelsz" in the area between 55°–58° N.Lat. and westward of 1° E.Long. in the North Sea in June and July of the years 1954, 1955 and 1957.

During each series of observations the ship proceeded at an average speed of 4–6 miles and remained in an area of 9 square miles. The light measurements were taken at deck level, during the hours of dusk and dawn. Once a day the extinction-coefficient of the total water column was measured. Thus we could calculate the depth of the levels receiving a given amount of light (*e.g.* 10, 5 or 1 lux) in the course of day and night. The line connecting the points receiving the same amount of light in the course of time is called here the iso-luxline. The steepness of these iso-luxlines can now be compared with the speed with which the herring schools rise and descend.

Observations and conclusions. All the bathythermograms had one thing in common: the surface temperature was always higher than the bottom temperature.

For our purpose we can distinguish four types of temperature gradients, the first three of which have actually been observed by us:

a. the temperature rising gradually form bottom till surface.

b. the temperature remaining either constant or rising but very gradually from the bottom till a given level, whereas the temperature gradient is rather steep in the higher water layers, up to the surface.

c. the temperature remains constant or rises but very gradually till a given level, next shows a rather sharp rise, but becomes equable again in higher water layers.

d. the temperature gradient showing a very sharp thermocline, between a rather homogeneous water temperature in the bottom layers and water layers with an equable temperature near the surface.

In comparing the behaviour of the herring schools with the temperature observations we found:

a. Unfortunately we did not observe any herring schools where such temperature conditions prevailed.

b. the schools came into the upper water layers, close to the surface.

c. the shoals ascended till the point where the temperature did not increase any more, in other words the schools just entered the layer of homogeneous surface water.

d. not observed by us, but SCHUBERT's observations demonstrated that the schools did not enter the surface layers of homogeneous water temperature in such a case.

The last three cases have in common that the schools rose till the point where the temperature did not further increase. In fact, pooling all our data, we find a very clear positive correlation between the level of the upper side of the schools and the depth of the homogeneous layer of warmer surface water. It seems justified to say now *that the depth of the herring schools at night depends on the vertical extension of the water layer of homogeneous temperature at the surface.*

Until now only the temperature observations have been considered. If we also take into account the isolux-lines (calculated from the light measurements in the course of dusk and dawn, in combination with the extinction coefficient measurements), it becomes quite obvious that the steepness of these lines corresponds roughly with the speed of the upward and downward movements of the herring schools. Our data further show that, if the herring follows a light optimum, this optimum must lie below the value of one lux, as measured by our method.

These observations support the hypothesis that light is an important factor in the causation of the diurnal vertical migration in the herring.

Our observations clearly demonstrate that two factors at least are involved in the pattern of the diurnal vertical migration in the herring, light and temperature.

The fact that the herring selects the layer which receives a certain amount of light shows clearly how light works as a regulating mechanism.

The role of water temperature is shown by the fact that the herring does not penetrate far into a layer of homogeneous temperature near the surface.

Though disagreeing at first sight, both SCHUBERT's and RICHARDSON's

observations fit into this scheme, if we may accept that in their cases the water layer, above the level to which the herring schools rose, were of a homogeneous temperature. But SCHUBERT's explatanion that the thermocline worked as a barrier does not receive any support from our observations.

The question remains why the herring does not rise to the surface in case of a superficial water layer of homogeneous temperature, whereas it does if the temperature rises and rises till the surface.

A possible explanation might be that the herring has both a certain light optimum, at a low level of light intensity, and a preference for rather high temperatures. Both factors working together will give the herring a stronger stimulus to rise than one factor alone. Possibly the herring has an aversion for coming into the neighbourhood of the surface, and it may be that if the temperature remains constant, the light factor alone is not strong enough a stimulus to cause the herring to rise close to the surface.

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2. A. B. STAM (Leiden). La dysentérie causée par les amibes chez les reptiles.

Un résumé de cette conférence n'a pas été remis au secrétaire.

3. F. H. SOBELS (Utrecht). Les effets nuisibles des radiations ionisantes sur le systeme génétique.

Voir: Vakblad voor Biologen, **38**, 169–183, 1958.

4. J. DE WILDE (Wageningen), aussi de la part de Mlle. C. S. DUINTJER, H. BONGA et L. MOOK. La photopériodicité chez les insectes.

Voir: Vakblad voor Biologen, **39**, no. 9, 1959.

5. Mlle. H. H. L. ZWILLENBERG. (Amsterdam). Die mikroskopische Anatomie der Milz der Furchenwale.

Milzstücke von Blauwal (*Balaenoptera musculus* (L.)), Finnwal (*Balaenoptera physalus* (L.)) und Seiwal (*Balaenoptera borealis* Lesson) wurden histologisch untersucht. Die Kapsel zeigt einen zweischichtigen Bau. Die äussere Kapselschicht, die den Hauptanteil der Kapsel bildet, ist arm an Muskelfasern und besteht zur Hauptsache aus dicken kollagenen Faserbündeln, die parallel zur Kapseloberfläche verlaufen. Die elastischen Fasern nehmen mengenmäßig von außen nach innen zu. Die innere Kapselschicht besitzt eine mäßig starke Muskulatur, die aus einigen Lagen sich rechtwinklig überkreuzenden Muskelfasern besteht. Die kollagenen Faserbündel und die elastischen Fasern sind hier feiner.

Die ganze Kapsel enthält viele Blutgefässe und außerdem große Nervenstämme, die von einer eigenen bindegewebigen Hülle umgeben sind. Außerdem finden sich viele freie Nerven, die von außen nach innen häufiger werden. Eine derartige zweischichtige Kapsel findet man ebenfalls bei den Huftieren, wo allerdings die Muskulatur stärker ausgebildet ist.

Die Trabekel sind gut entwickelt und verzweigt. Die kollagenen Faserbündel verlaufen im allgemeinen in der Längsrichtung des Balkens. Die elastischen Fasern sind nicht so streng ausgerichtet. Die Muskulatur ist mäßig entwickelt. Die Muskelfasern verlaufen hauptsächlich in der Längsrichtung der Trabekel. Das Muskelgehalt schwankt stark, auch innerhalb eines Balkens. Die Trabekelmuskulatur der Huf- und Raubtiere ist vergleichsweise viel stärker entwickelt und gleichmäßiger verteilt. Die Trabekel werden von vielen in der Längsrichtung des Trabekels verlaufenden Nervenfasern innerviert. Außerdem trifft man häufig Trabekel mit einem zentralen Nervenstrang an. Ein peritrabekulärer Plexus fehlt. Die Trabekel, die ausschließlich Arterien enthalten, sind häufiger als Trabekel mit Venen. Die Trabekelarterien besitzen öfters eine sehr stark entwickelte Tunica intima. Solche "Polsterarterien" wurden vor allem beim Seiwal angetroffen. Die großen Trabekelarterien werden meistens von einem bis mehreren großen Nervenstämmen begleitet. Nur die sehr grossen Trabekelvenen besitzen eine eigene Wand. Meistens ist aber die Trabekelvene als ein aus Endothel bestehendes Rohr in das Balkengewebe eingelassen. Die Innervation ist hier weniger intensiv. Solche Trabekelvenen werden auch bei einigen Huf- und Raubtieren beschrieben.

Die weiße Pulpa konzentriert sich hauptsächlich auf die Malpighi'schen Körperchen. Nerven wurden nur sporadisch an der Peripherie der Malpighi'schen Körperchen wahrgenommen. Mit der Papierwägemethode wurden einige quantitative Bestimmungen der weißen Pulpa durchgeführt. Für den Finnwal wurde ein mittlerer Wert von 13,2% der gesamten Schnittfläche gefunden, für den Blauwal 18%, für ein Exemplar des Seiwales 14% und für zwei weitere Exemplare 30%.

Die rote Pulpa besteht zur Hauptsache aus weitmaschigem retikulärem Bindegewebe. Eine Pulpamuskulatur fehlt. Kapillare mit Scheiden von Schweigger-Seidel und venöse Sinus wurden nicht angetroffen. Letztere fehlen ebenfalls bei den Huftieren und den meisten Raubtieren. Auffallend waren die vielen großen Anschnitte von Pulpavenen, deren Wand nur aus Endothel besteht, das von wenig Retikulum umsponnen wird. Die Innervation der roten Pulpa besteht aus wenigen in der Pulpa frei verlaufenden Nervenstämmen und vielen feinen Nervenfasern. Das meistens feinkörnige Hämosiderin liegt überwiegend

extrazellulär in der roten Pulpa zerstreut. Auffallend ist der große Reichtum an eosinophilen und neutrophilen Granulozyten. Auch Plasmazellen kommen in großer Zahl vor. Diese Zellen können die sogenannten RUSSEL-Körperchen enthalten, die PAS-positiv sind. Die verschiedenen Stadia der Entstehung, des Zusammenfließens und der Ausschüttung in die rote Pulpa wurden an Hand von Diapositiven demonstriert.

Anschließend wurde zur Diskussion gestellt, ob die Milz der Furchenwale als eine "Speichermilz" oder als eine "Abwehrmilz" gesehen werden muß. Diese Begriffe wurden von VON HERRATH geprägt. Eigenschaften wie das Überwiegen der roten Pulpa über die weiße, das kräftig entwickelte Kapsel- und Trabekelsystem, sowie das Fehlen der Sinus könnten darauf hinweisen, daß er sich hier um eine Speichermilz handelt. Die geringen relativen Maße und das geringe relative Gewicht des Organes lassen aber an einer effektiven Speicherfunktion Zweifel aufkommen. VON HERRATH faßt die Speicherfunktion als eine Speicherung und Bereitstellung einer unveränderten Blutreserve auf. Rezente Untersuchungen haben aber deutlich gezeigt, daß sich das Blut während der Speicherphase qualitativ verändert. Auch wurde gezeigt, daß nicht die Menge des abgegebenen Milzblutes wichtig ist, sondern dessen Qualität. Die Interpretation der Speicherfunktion hat sich also verschoben. Außerdem zeigten einige Autoren, daß die rote Pulpa eine ebenso große, wenn nicht größere Rolle bei der Abwehr spielt als die weiße Pulpa. Deshalb ist der Begriff Abwehrmilz, der sich auf ein Überwiegen der weißen Pulpa stützt, mit den heutigen Auffassungen nicht mehr in Übereinstimmung. Eine physiologische Interpretation quantitativer morphologischer Merkmale, wie sie in den Begriffen Speichermilz und Abwehrmilz zum Ausdruck kommt, sollte vermieden werden. Die rein morphologische Einteilung von SNOOK wird deshalb vorgezogen, nach der die Milz der Furchenwale als eine Retikulummilz mit primordialen Venen ohne Hülsenarterien klassifiziert wird. Aus den Untersuchungen ging hervor, daß die in der übrigen Anatomie der Wale stets auftretende Kombination von Huf- und Raubtiermerkmalen auch in der Milz der Furchenwale wiederkehrt. Trotz der geringen relativen Größe macht das Organ keineswegs einen rudimentären Eindruck.

6. Mlle. H. M. Th. VAN TURNHOUT (Amsterdam). Control of *Lygus campestris* in carrot seed crops in North Holland.

Voir: Tijdschr. over Plantenziekten, **64**, 301–306, 1958.

Séance du 22 février 1958 à Amsterdam

P. KORRINGA (IJmuiden). *Mytilus* et *Mytilicola*, l'interaction entre hôte et parasite.

Voir: Vakblad voor Biologen, **31**, 63–74, 1951; Visserijnieuws, **3**, no. 7, suppl.; Conseil Perm. Expl. Mer Ann. Biol., **8**, 182–185, 1951; *ibid.*, **9**, 219–225, 1952; *ibid.*, **10**, 197–202, 1953; *ibid.*, **11**, 184–186, 1954; *ibid.*, **12**, 230–232, 1955.

Séance du 22 mars 1958 à Utrecht

1. P. C. Heyligers (Utrecht), aussi de la part de P. Glas et J. J. Beukema. The shrimp's reaction to the height of the water.

The shrimps inhabiting the Wadden Sea during summer show tidal migration. When the tide comes in, they invade sand bars that are dry at low tide, but they return to the deeper channels when the tide falls. Only a few young individuals allow themselves to be stranded on the sand bars. The problem of how the shrimps perceive that the tide is falling has been subjected to an experimental analysis at the Zoological Station in den Helder.

It was found that it is not the decrease of hydrostatical pressure at the bottom which induces the shrimps to move away. However, visual stimulation by the falling water level suffices to make a burrowed shrimp come out of the sand. No perception of the water surface by the antennae is involved. In the dark shrimps can determine the depth of the water without the aid of either eyes or antennae. Movements of antennulae, antennae and cephalothorax precede the shrimps' departure. The shrimps must be in good condition for the experiments to be successful. The movements of the antennulae and the manner of burrowing provide an index of the animal's condition. The experiments are being continued, in combination with field observations.

2. J. J. W. de Blok (den Helder). Un appareil pour l'étude de l' influence de la périodicité lunaire sur les animaux marins.

Un résumé de cette conférence n'a pas été remis au secrétaire.

3. C. R. Janssen (den Helder). Reversal of geotaxis and fototaxis induced in *Littorina obtusata* by temperature changes.

Voir: Arch. néerl. zool. (this issue).

4. J. Verwey (den Helder): Orientation in migrating marine animals.

Voir: Arch. néerl. zool., **13**, suppl. 1, 418–445, 1959.

Séance du 3 mai 1958 à Amsterdam

1. D. Stegwee (Wageningen).

Un résumé de cette conférence n'a pas été remis au sécrétaire.

2. H. Klomp (Wageningen). Considérations théoriques sur le rôle des parasites entomophages dans l'écroulement d'une calamité des insectes.

Voir: Arch. néerl. zool., **13**, 134–145, 1958; Proc. xvth Int. Congr. Zool. London, 1958.

3. H. N. Kluijver (Arnhem). Roosting habits, sexual dominance and survival in the Great Tit.
Voir: Cold Spring Harbor Symp., **22,** 1957.

4. J. Faber (Utrecht). Fore limb regeneration in the Axolotl: prospective significance and self-differentiation of the blastema.

In 1939 Mettetal discovered that early limb regeneration blastemata of Urodeles, transplanted in a "neutral" environment, formed only distal limb structures (fingers). Increasingly older blastemata formed more and more proximal structures in addition.

Mettetal explained his results by assuming that the blastema grows at the basis by immigration of cells from the limb stump. Consequently the mesenchyme of the early blastema would ultimately come to lie at the distal end of the regenerate. From the beginning the determination (and self-differentiation) of the different regions of the mesenchyme would be in accordance with their prospective significance. We would thus have to do with a mosaic, to which during the process of regeneration more and more parts would be added, determined to form more and more proximal structures.

We have extended Mettetal's investigations, using *Amblystoma mexicanum*, but we have also carried out a number of carbon-marking experiments. The latter have revealed that the mesenchyme of the blastema does not grow at its basis, but primarily at its tip, just as was shown by Tschumi (1955) for the embryonic hind limb bud of *Xenopus*. Consequently the prospective significance of the mesenchyme of the early blastema is the proximal region of the limb, a fact which invalidates Mettetal's explanation. Prospective significance and determination (self-differentiation) of the early blastema do not coincide.

For the transplantation experiments larvae of 7–10 cm long were used. The fore limbs were amputated through the middle of the upper arm. Blastemata of four successive stages were transplanted autoplastically to the flank, midway between the fore and hind limbs, hence outside both "limb territories", as defined by Guyénot *c.s.* In the transplants the skeletal elements differentiated as follows: stage I (small conical blastema) formed only finger elements (*i.e.* phalanges and metacarpals; these cannot be distinguished in the transplants); stage II (larger conical blastema) sometimes also formed carpal elements; in transplants of stage III ("paddle" stage) sometimes one or two zeugopodal elements (radius and/or ulna) appeared in addition, while stage IV (first finger indicated) sometimes showed the differentiation of an additional stylopodal element (humerus). These results are in agreement with those of Mettetal.

Besides entire blastemata also proximal and distal halves of blaste-

mata of stage IV were transplanted. Both the distal and the proximal halves showed skeletal differentiation of a markedly distal type (finger elements with or without carpus). The proximal portion of the new humerus, already present in the form of procartilage in the proximal half, disappeared completely from the transplants. Apparently all this is the result of the presence of a wound surface at the distal end of the proximal half.

Five regional categories of limb structures were distinguished, *viz.*: 1. the fingers as morphological entities, and 2. to 5., the skeletal elements of respectively the fingers, the carpus, the zeugopode and the stylopode. The differentiation of these categories may be expressed in percentages of the numbers of corresponding structures found in the normal limb. These percentages may be denoted as "manifestation degrees". It appears that in all experiments invariably the fingers as such show the highest average manifestation degree, the other categories showing decreasing manifestation degrees in a disto-proximal direction. This decrease becomes less and less steep according as more and more proximal levels of differentiation are represented in the transplants concerned.

For the explanation of the above phenomena the following hypothesis is advanced: the mesenchyme of the early blastema possesses intrinsic differentiation tendencies, leading to distal differentiation only. In the proximal portion of the blastema, later more proximal differentiation tendencies appear under the influence of the limb stump. The distal tendencies present in the distal portion, nevertheless remain relatively stronger. The most proximally situated, at the same time "oldest", mesenchyme goes through the complete range of differentiation tendencies, from intrinsic or autonomous distal ones to entirely dependent proximal ones. In the isolated proximal half of the blastema of stage IV, under the influence of the new distal wound surface dedifferentiation would occur, attended with a disappearance of dependent proximal differentiation tendencies, and a reappearance of autonomous distal tendencies.

It is of interest to note that an obvious parallel exists between the hypothesis advanced here, and the "activation-transformation" hypothesis put forward by Nieuwkoop *et al.* (1952) with regard to the neural induction process in Amphibia. The intrinsic distal differentiation tendencies of the mesenchyme of the early regeneration blastema might be compared with the intrinsic prosencephalic differentiation tendencies appearing in the neuro-ectoderm upon "activation", whereas the more proximal tendencies appearing under the influence of the stump in mesenchyme which previously only possessed distal tendencies, might be compared with the more caudally directed differentiation tenden-

cies appearing in activated ectoderm under the "transforming" influence of the archenteron roof.

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TSCHUMI, P. A., 1955: Versuche über die Wachtstumsweise von Hinterbeinknospen von *Xenopus laevis* Daud. und die Bedeutung der Epidermis. Rev. Suisse Zool. **62,** 281–288.

5. J. J. LAARMAN (Leiden). L'entomologie et la lutte moderne contre la malaria.

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 11 octobre 1958 à Leiden

1. H. J. VONK (Utrecht). Investigation of metabolism by means of isotopes.

Before 1900 the way in which the conversion of carbohydrates, fats and proteins to carbondioxide, water and some nitrogen compounds (like urea) is taking place was unknown. Since then the dissimilation of glycogen to lactic acid and pyruvic acid and further on to acetate, the combustion of acetate in the TCA cycle to CO_2 and H_2O and the part which is played in this process by the cytochrome-system have been elucidated. The deamination and transamination of amino acids has been discovered. Many of these catabolic processes are reversible and may lead to syntheses. In lower vertebrates and invertebrates our knowledge of all these processes is still extremely limited.

In obtaining these results, since 1934 the use of isotopes has played a very important part. At first stable isotopes like H^2 (deuterium), C^{13} and N^{15} were used. They must be determined by means of the mass spectrometer (MS) which is expensive and cumbersome. So it is a great advantage that at present the radioactive C^{14} is available which can be determined much more easily and far cheaper by means of a GEIGER-MÜLLER counter. Instead of deuterium (H^2) tritium (H^3) can now be used, but because of its weak radiation not without difficulty. Radiotopes of nitrogen are useless because of their short half-life, so that the stable N^{15} provides the only possibility for working with nitrogen-labelled compounds. Deuterium is still useful because it can be determined also by other means than by the MS. Isotopes of nearly all elements can be applied, but not all of them are commercially available.

Compounds like acetate, higher fatty acids, glucose, amino acids, water and phosphate, which play an important part in the interme-

diary metabolism may be "labelled" with isotopes. If animals take up these labelled compounds with their food or by injections, they take part in the metabolic processes in exactly the same way as the corresponding unlabelled compounds. By finding out to which other compounds the isotopes are transferred, conclusions can be drawn concerning the metabolic pathways in the organism.

A good recent example is the investigation of the metabolism of cholesterol. By feeding acetate-1-C^{14} and (or) acetate-2-C^{14} it has been found that all the 27 C-atoms of cholesterol may originate from acetate and which of them have their origin in the C-1 or C-2 atoms of this acetate. As intermediates between acetate and cholesterol mevalonic acid and squalene have been found. Cholesterol (if supplied labelled) has been found to be the precursor of bile acids and steriod hormones. The way back from these to cholesterol seems to be impossible. Neither is cholesterol used for production of energy (*e.g.* by degradation to acetate).

In invertebrates the principal sites for syntheses of different compounds can be determined by feeding or injecting D_2O. Injection of labelled compounds can also serve for determining the essential dietary requirements of animals in cases where feeding experiments are impossible (aquatic animals, phytophageous insects).

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WAARD, A. DE, 1958: Investigations on the intermediates in the biosynthesis of fatty acids and cholesterol. Dissertation Utrecht.

2. P. D. J. W. SEDEE (Utrecht). Intermediary lipid and amino acid metabolism in germ-free bluebottle larvae.

Since the early use of labelled compounds in metabolic studies an impressive body of knowledge has accumulated about the intermediary metabolism of mammals, birds, and bacteria. On the other hand not much attention has been paid to these processes in invertebrates and lower vertebrates and mostly it is assumed that their metabolic processes might follow the same patterns as that found in mammals and (or) bacteria. Only in the last years some contributions in this field have been made, *viz.*: *Helix*, *Periplaneta*, (VONK *et al.*, 1956), *Bombyx* (BRICTEUX-GRÉGOIRE *et al.*, 1956; FUKUDA, 1956), *Calliphora* (SEDEE 1956, 1958), *Periplaneta* (CASIDA *et al.* 1957).

In this paper the results of a study of the intermediary metabolism of an insect are given. In studying metabolic processes in invertebrates, bacteria in the gut and possible endosymbionts might cause ambiguous results. Therefore an insect was chosen that was free from endosymbionts and moreover ccn be reared under aseptic conditions, *viz.:*

the larva of the bluebottle, *Calliphora erythrocephala*. Another reason was that from previous experiments a fair knowledge of its dietetic requirements and of its amino acid metabolism had been obtained (SEDEE 1956). In these experiments it was found that this carnivous larva can be reared under aseptic conditions on pure casein supplemented with cystine, B-vitamins, cholesterol, salts, and water. Any addition of fats or carbohydrates to this food did not result in a better growth. But, larvae reared from the egg on this food contained a fair amount of fats and carbohydrates which shows that they are able to synthesize these compounds from amino acids or their breakdown products. Since it was found that in the metabolism of mammals and bacteria acetic acid (as acetyl-coenzyme A) is a common intermediate in the metabolism of fatty acids, cholesterol, diverse amino acids, and carbohydrates, sodium acetate-1-C^{14} seemed to us a suitable labelled compound to use in these experiments.

About 2300 larvae were reared under aseptic conditions on 1360 g of the aforementioned food to which 2.98 g $CH_3C^{14}OONa$ (specific activity 53 μC per gram) was added. After 6 to 7 days the larvae were full-grown. They were then killed and dried. The dried larvae were extracted with ether and from the residue of this ether extract cholesterol and fatty acids were isolated. The extracted larvae were hydrolyzed with HCl and a number of amino acids isolated and purified. Samples of the food, the added acetate, and of the isolated compounds were converted to CO_2, by dry or wet combustion and collected as $BaCO_3$. All these samples of $BaCO_3$ were washed, dried, and plated at infinite thickness on aluminium-disks having a constant area, and were counted with a thin-window counter.

The following results were obtained: The isolated cholesterol did not show any radioactivity, which shows that no synthesis of this compound occurred during larval life. This is in good agreement with the findings in dietetic studies. Furthermore this finding excludes the possibility of synthesis of cholesterol in the larvae at such a low rate that they cannot meet their demands of normal growth.

The crude mixture of fatty acids showed a fair radioactivity. From this mixture the higher saturated fatty acids (C_{12} to C_{24}) were isolated and separated. It was found that by far the largest amount of the saturated fatty acids consisted of palmitic acid (C_{16}) which also showed the highest radioactivity among these fatty acids. When the intermediary synthesis of a fatty acid takes place by β-condensation from $CH_3C^{14}OOH$, the following acid arises $CH_3C^{14}H_2CH_2C^{14}H_2$........ ...$CH_2C^{14}OOH$. Therefore the activity of the carbon derived from the COOH must be about twice that of the total carbons derived from the fatty acid. In order to determine if palmitic acid was syn-

thesized by β-condensation from the fed acetate, the radioactivity of the carbon of its COOH group was determined. This was found to be about equal to the total radioactivity of the palmitic acid. Whether β-condensation plays a role in the intermediary synthesis of palmitic acid cannot be decided from the results of this experiment; anyhow no direct use of the fed labelled acetate has been made.

The following amino acids were isolated: glutamic acid, alanine, aspartic acid, *lysine*, *histidine*, *arginine*, *valine*, *leucine*, proline, serine, glycine, *phenylalanine*, and tyrosine. Among these amino acids the essential ones (in italics) do not show any significant radioactivity. This finding was to be expected since the essential nature of these amino acids is due to the fact that the animal is unable to synthesize their carbon chains. All the non-essential amino acids, except tyrosine, showed radioactivity. The absence of radioactivity in tyrosine, shows that this amino acid, like in mammals, is formed by oxidation of the essential phenylalanine.

On the basis of these results a rapid and easy method for studying the qualitative amino acid requirements in animals is suggested. A suitable C^{14}-labelled compound is administered to the animal for a certain period, and then its amino acids are isolated and checked for radioactivity. Absence of radioactivity in an amino acid (with the exception of tyrosine) shows that the amino acid is essential. With the aid of this method dietetic studies can be avoided. This will be of importance for the study of animals with specialized feeding habits and for aquatic animals, where dietetic studies are very difficult.

Among the non-essential amino acids the highest radioactivity was found in glutamic acid, alanine, and aspartic acid, three amino acids whose keto acids plays an important role in the KREBS cycle. Since acetic acid as acetyl-coenzyme A enters this cycle, the C^{14} in these amino acids was to be expected.

In the metabolism of the rat glutamic acid, proline, ornithine, and arginine are mutually interconvertible (ROLOFF *et al.* 1940; WOMACK and ROSE 1947; STETTEN 1951). With the aid of degradation studies of the isolated glutamic acid and proline, it was found here that some proline was formed from glutamic acid, but this process seems not to play a very important rôle in the metabolism of the larvae. No arginine was formed from glutamic acid.

Degradation studies of the isolated glycine and serine, in which the activity of each carbon was determined separatedly, showed that these two amino acids can be converted into each other, as has been found in the metabolism of the rat.

The results of these studies show that the intermediary amino acid metabolism of the larvae is about the same as that found in mammals.

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WOMACK, M. and W. C. ROSE, 1947: J. Biol. Chem., **171**, 37.

3. W. COMPANJEN (Wageningen), Histologie des cellules neurosécrétoires et des organes endocrines de *Bupalus piniarius* L. pendant la métamorphose.

Un résumé de cette conférence n'a pas été remis au secrétaire.

4. J. VAN DER VECHT (Leiden). Zoological Nomenclature at the International Congress of Zoology, London 1958.

A report of the discussions held during the Colloquium on Zoological Nomenclature preceding the xvth International Congress of Zoology at London in 1959. A more extensive paper on current trends in zoological nomenclature will appear in this journal after the publication of the Resolutions agreed on in London in the form of a new code of Rules of Nomenclature.

Séance du 14 novembre 1958 à Maastricht

1. P. J. VAN NIEUWENHOVEN (Maastricht). Les fosses de marne du Limbourg du sud et leurs chauve-souris.

Voir: VAN NIEUWENHOVEN, Diss. Amsterdam 1956; PUNT and VAN NIEUWENHOVEN, Experientia, **13**, 51–54, 1957; DE WILDE and VAN NIEUWENHOVEN, Pub. Nat. Hist. Genootsch. Limburg, Ser. VII, 51–83, 1954.

2. A. VAN WIJNGAARDEN (Bilthoven). A census of the badger, (*Meles meles* (L.)) in the Netherlands.

Both for the correct management of existing State nature reserves, and for drawing up a well planned acquisition scheme for new reserves, a geographical-ecological census of all rare and threatened species is wanted.

One of the first mammal species studied by the R.I.V.O.N. (State Institute for Nature Conservancy Research) is the badger, *Meles meles* (L.), because this big predator is diminishing rapidly.

During a thorough study of the literature and of Dutch hunters' periodicals many data were collected on its food habits, natality, burrows, home range, *etc.* Its former range in the Netherlands was reconstructed.

In a field census following this, all badger burrows known to game keepers, farmers, rural policemen, *etc*,. were visited, mapped and described.

This work was finished in the spring of 1959, the results will be published soon.

3. J. Th. ter Horst (Maastricht). Les reptiles et les amphibies dans le Limbourg du sud.

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 13 décembre 1958 à Amsterdam

1. N. Tinbergen (Oxford). The adaptive radiation of signalling movements in gulls (Laridae).

Comparative studies of the displays of various gulls (the large gulls—Herring Gull and close relatives—, the hooded gulls—Black-headed Gull and relatives—and the Kittiwake) by members of the Animal Behaviour Unit of the Dept. of Zoology, Oxford (C. Beer, J. M. Cullen, E. Cullen, G. Manley, M. Moynihan, N. Tinbergen, R. Weidmann, U. Weidmann) and others have led to the following results.

The displays of gulls are relatively constant within each species, and can be compared in the same way as structures are compared in anatomy.

There is a striking overall similairty of the display repertoires of the species studied, which strengthens the conclusion that the gulls are a monophyletic group.

There are many consistent differences between the species. Functional studies show that many of these differences are adaptive.

Analyses of the forms of the displays and of the causation underlying them show that they are the outcome of dual or multiple motivation. When this evidence is combined with comparative evidence, the probable origin of several displays can be traced.

The adaptive evolution of the displays seems to have been directed by four types of selection pressure. (1) Direct pressure towards increased conspicuousness (rhythmicity, exaggeration or reduction, colourful structures, speed, freezing). (2) Indirect intraspecific pressure towards inter-display distinctness. (3) Direct pressure towards interspecific differences promoting sexual isolation. (4) Indirect pressure resulting in inter-specific distinctness. Sexual isolation may be, in some cases, the consequence of (4) rather than of (3).

The existence of convergences in displays is emphasised.

A detailed report will appear in "Behaviour".

2. Mlle. E. C. Boterenbrood (Utrecht). Organization in aggregates of anterior neural plate cells of *Triturus alpestris*.

Voir: Proc. Kon. Ned. Akad. Wet. Amsterdam, C, **61,** 470–481, 1958.

3. A. C. POSTHUMUS (Leiden). The correlation between some measurements of the brain in some birds.

The brain is an important factor in determining the shape of the entire head and the skull. Reversely, the shape and the structure of the brain depend on the function, which the brain has to exercise and the position it takes in the head.

The aim of this research was to determine, first, the structural relation between the different brain parts and, secondly, the connection of this relation with the function and the position in the entire head. A number of measurements of the different brain parts were taken. The preliminary results can be summarized as follows:

The maximal length of the total brain is directly proportional to the maximal width of the cerebellum and of the telencephalon. However, the investigated birds can be divided into two groups:

1. birds with a relatively long telencephalon and a short cerebellum.
2. birds with a relatively short telencephalon and a long cerebellum.

In both groups the relation between the length and the width of the telencephalon is directly proportional, this relation exists also in the cerebellum.

In small birds the cerebellum and the telencephalon are relatively higher than in big ones.

On the basis of these facts it is expected that, concerning the volume of the different brain parts, the birds can be divided into four groups. Volume measurements did not show this division clearly; there was only a small indication.

Whether the difference in relative size of the various brain parts can be correlated with certain functions or not could not yet be established. There is a vague indication in the direction of a functional relation.

The rate of growth is higher in the telencephalon than in the cerebellum. Of the cerebellum the growth in lateral direction is relatively slower than in the antero-posterior direction.

4. Mlle. J. R. GOUDAPPEL (Amsterdam). Microscopic structure of the lungs of the Bottlenose Whale.

Voir: GOUDAPPEL, J. R. and E. J. SLIJPER, Nature, **182**, 479, 1958.

5. P. G. W. J. VAN OORDT (Utrecht), aussi de la part de L. BASU. The influence of testosterone on the spermatogenesis of the common frog.

Prolonged treatment of male common frogs *(Rana temporaria)* with testosterone, administered as hypodermic pellets, results in complete blockade of spermatogenesis if the treatment has been started before or at the beginning of the spermatogenetic activity. Testosterone has either no effect upon the maturation divisions and spermiogenesis, or only slightly accelerates these processes.

It has been found (P. G. W. J. van Oordt, Thesis Utrecht, 1956) that hypophysectomy also impedes the mitotic activity of the secondary spermatogonia, and not the transformation of spermatocytes into sperm cells via spermatids.

The action of testosterone, therefore, may be mainly confined to an inhibition of the gonadotropic activity of the pituitary gland.

Experiments are in progress to verify this hypothesis.

Séance du 24 janvier 1959 à Utrecht

1. P. R. Wiepkema (Groningen). A motivation analysis of the courtship behaviour of the bitterling (*Rhodeus amarus* Bloch).

In spring the males of the bitterling defend areas around a mussel (*Unio*, *Anodonta*). Other males are chased away, but females with long ovipositors are guided towards the mussel. During this leading the male quivers with its entire body. The females deposit their eggs within the gills of the mussel by means of the long ovipositor. Before and after egg laying the male makes skimming movements over the siphons of the mussel. Simultaneously with skimming the male may eject sperm. Through the inhalent siphon ot the mussel this sperm reaches the eggs in the gills.

In the behaviour of the male of the bitterling several movements and postures can be distinguished. The question arises how far these behaviour elements have causal factors in common. I have especially examined their interrelationship during the reproductive period. As exactly as possible the behaviour of 13 different territorial males was recorded, in the presence of another male as well as with a ripe and an unripe female. From the data of these observations one can compute whether or not a certain movement precedes or follows each of the other movements more or less frequently than one would expect if they are not causally interrelated. Consequently positive and negative correlations can be distinguished respectively. On account of this sequence-analysis I found 3 separate groups of movements. The movements of a group are characterised by a mutual positive correlation, whereas there is a clear negative correlation with all other movements.

The 3 groups are:

a. Tailwagging, turning beat, head butting, chasing, fleeing (agonistic behaviour).

b. Quivering, quivering with leading, head down posture on the mussel, skimming (sexual behaviour).

c. Feeding, chafing, finclipping (feeding and cleaning movements); because these movements are not typical for the reproductive period I have left them out of consideration.

To come to a better understanding of the interrelations of these different movements special attention was given to the striking behaviour changes of the male before and after egg laying. Before egg laying one seldom finds any agonistic behaviour in the male. However, immediately after egg laying there is a strong increase of aggressive behaviour, lasting for some minutes. Before egg laying the sexual movements—quivering, skimming and sperm ejection—have a rather high and constant value; after egg laying, however, quivering and sperm ejection show a distinct drop for some minutes, whereas on the contrary skimming shows a temporary increase. These changes are remarkable as in all other situations the frequencies of quivering, sperm ejection and skimming show a clear positive correlation.

In order to solve this problem I examined the influence of the activation of the aggressive system on quivering, sperm ejection and skimming. In a number of experiments I recorded the behaviour of a territoral male in the presence of a ripe female immediately after a period of strong aggression. After such a period the tendency to show aggressive movements is increased. It was clear that a higher tendency to perform aggressive movements goes together with a decrease in the frequencies of quivering and sperm ejection, but the frequency of skimming does not change.

These facts may be interpreted by assuming that:

a. Aggression *and* sexual behaviour increase after egg laying.

b. Quivering and sperm ejection are inhibited by the strong aggression.

c. Skimming is not or hardly influenced by the same aggression.

It is very likely that the stimulation of the aggression after egg laying is caused by the smell of the newly layed eggs. This aggression is certainly not caused by sperm ejection as sperm is also ejected before egg laying when no aggression is seen.

2. L. D. Brongersma (Leiden). Recherches zoologiques dans la Nouvelle-Guinée Néerlandaise.

Un résumé de cette conférence n'a pas été remis au secrétaire.

3. D. A. Hooyer (Leiden). Rongeurs fossiles et récents des Iles Antilles Néerlandaises.

Voir: Studies fauna Curaçao, **9,** 1–27, 1959.

4. P. H. J. van Bree (Amsterdam). "Homing" chez *Patella.*

Voir: Versl. Kon. Ned. Akad. Wet. Natuurk., **68,** no. 7, 1959.

5. H. J. Nieboer (Groningen). Ethological observations on the ant-lion *(Euroleon nostras* Fourcroy, Neuroptera).

Discussions on the behaviour of the ant-lion were for a long time centered around the question: Is behaviour adaptive (plastic) or rigid? Doflein (1916) in his monograph asserted that the behaviour

of the ant-lion is composed of three main reflexes ("Grundreflexe"): "Einbohrreflex", "Schleuderreflex", "Schnappreflex" and some other less important reflex-movements. The conclusion of his reflex-biological study was: "Solange das Tier Larve ist, ist es ein reiner Reflexautomat, es funktioniert wie eine kleine Maschine."

Doflein's criticists (Meissner 1917, 1919; Stäger 1924, 1925; Bierens de Haan 1925 a.o.) assembled facts and anecdotes to support the view, that the behaviour of the ant-lion is plastic and adaptive. Ethological research on other animals, however, showed that this kind of controversy is mainly due to one party studying the animal under one and the same, and the other party under several different internal conditions ("Stimmungen", "motivations"). The uniformity of Doflein's results can be explained by the fact that in his experiments he studied the behaviour of the ant-lion outside its normal environment (dry sand), i.e. with the animal in flight-motivation.

In our study attention was mainly paid to the following points: *a*. Description of behaviour-elements; *b*. Causation of these elements, particularly with regard to common causal factors (analysis of instinct-hierarchy).

About thirty behavioural elements can be distinguished, belonging to different groups, e.g. digging the pitfall; capturing and eating (sucking) the prey; pushing pebbles out of the pit; migration and others. The statement of Doflein (1916), Macgillavry (1936), Eglin (1939), Berland (1949,) that the differences between their observations of the pitmaking behaviour and the observations of older authors *e.g.* Rösel von Rosenhof (1755), may be reduced to a difference of species or of their observational conditions, does not fit. The species concerned (*Euroleon nostras* F. and *Myrmeleon formicarius* L.) show the same digging behaviour (Bakker 1943). In my opinion all differences in this behaviour described between these species must be attributed to keeping the animals under conditions that were insufficiently adequate and standardized for comparative purposes. Differences in the amount of background noise or the degree of satiation I found to be of paramount influence on the tendency to dig. Throwing sand at a potential prey is released by vibrations from a prey moving on or near the slope of the pit (Rabaud 1927). The insect throws sand in only two directions with respect to its body, namely to the left or to the right. These movements can be made separately or in bursts.

Sand is thrown almost exclusively to one side if the prey is placed laterally to an ant-lion. It is thrown to the right and left alternately in the following cases: (1) when the ant-lion is stimulated dorsally; (2) when it is stimulated frontally; (3) at simultaneous stimulation on both sides of the animal; (4) when a lateral stimulus is given at a relatively

great distance; and (5) when stimulated by a sound produced in the air (± 500 Hertz, LE FAUCHEUX 1958).

If a stimulus releasing a number of responses is followed, after an interruption of less than 1 min., by a renewed stimulus, the first response of the resulting burst is given to the side contralateral to that of the last response in the preceding burst. This is independent of whether the order of the stimuli is frontal–frontal–frontal *etc.*, dorsal–dorsal–dorsal *etc.*, frontal–dorsal–frontal–dorsal *etc.*, or lateral–contralateral–lateral–contralateral *etc.*

When digging, the ant-lion performs alternate throwing movements which, although they seem to consist of the same co-ordinations as throwing at prey, are clearly distinguishable from the latter—for instance in the speed and position of the mandibles. This type of alternate throwing occurs in the following cases: (1) when finishing the trapping pit; (2) after some sand has fallen into the pit; (3) after flinging away a sucked prey.

The occurrence of alternating sequences both in sand-throwing at prey and sand-throwing for digging, suggest that their taxis components have principles of co-ordination in common.

Interesting results are obtained when the number of responses to the left and the right are compared in two types of experiments in which the animal is stimulated either in the order dorsal–lateral–contralateral, or lateral–contralateral–dorsal, in such a way that the switch to another place of stimulation is made only after the responsiveness to stimulation at the former place has faded out to a given low level. At the end of both types of experiments the animals are no longer able to react, but those of the series dorsal–lateral–contralateral have given about double the number of responses given to the other. The former series gave responses in equal numbers to the left and to the right, the latter threw sand about as often to the side first stimulated, but very much less to the contrala-teral side. It seems that the insect is able to perform only a certain amount of reactions to one side but that this responsiveness can be very much exhausted by stimulating the other side and/or reacting to these stimuli.

An animal no longer able to react to a series of ten lateral stimulations, even not after a resting-time of two minutes (i.e. the time necessary for ten stimulations) will usually react at once when stimulations are applied to the dorsal side. Immediately after these dorsal stimulations (reactions) it reacts again on lateral stimulation.

Séance du 21 février 1959 à Amsterdam

D. DRESDEN (Utrecht). "Insectoxicologie?"

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 11 avril 1959 à Groningen

1. A. P. VAN OVERBEEKE (Amsterdam). Sur l'histologie du tissu interrénal et du tissu chromaffine chez quelques poissons téléostéens.

Chez les Téléostéens le tissu interrénal est situé dans la région lymphoïde des reins céphaliques. C'était GIACOMINI qui, dès 1908, a étudié cette structure épithéliale minutieusement. D'après ses observations on trouve dans la plupart des Téléostéens le tissu interrénal sous forme de îlots glandulaires plus ou moins irréguliers autour des veines cardinales postérieures droite et gauche. GIACOMINI a signalé aussi que, dispersé entre les îlots mentionnés ci-dessus, il y a des groupes de cellules qui, après fixation dans le liquide de Müller, montrent dans leur cytoplasme une nette phaeochromie. En 1928 BAECKER a décrit les résultats d'une récherche de ces tissus épithéliaux. Il a pu confirmer les données de GIACOMINI. Comme lui il distingue deux types de cellules, mais quant à la phaeochromie, il ne voit se produire ce phénomène qu'irrégulièrement, un fait qui, à son avis, doit être attribué aux difficultés de la technique de fixation.

Aujourd'hui on constate un nouvel intérêt aux "organes interrénaux" des Téléostéens, surtout du côté des endocrinologistes. Les études histophysiologiques de RASQUIN (1951), de PICKFORD (1953), de CHAVIN (1954, 1956) et de OLIVEREAU et FROMENTIN (1954) ont montré que chez les Téléostéens eux aussi, le tissu interrénal est sous le contrôle de l'hypophyse.

Nous avons étudié les tissus épithéliaux des reins céphaliques chez quelques espèces de Téléostéens, c-à-d: *Betta splendens* Regan, *Lebistes reticulatus* Peters, *Gasterosteus aculeatus* L., *Esox lucius* L., *Cyprinus carpio* L., *Carassius auratus* L., *Perca fluviatilis* L., *Leuciscus rutilus* L. et *Cottus scorpius* L.

Chez toutes ces espèces le tissu épithelial s'est composé de deux types de cellules.

a. L'un forme dans la majorité des cas des cordons épithéliaux entourant les veines cardinales postérieures et ses ramifications. En général ce tissu n'est séparé de la lumière vasculaire que par un mince endothélium. Le cytoplasme est finement granuleux mais parfois, comme par exemple chez *Carassius auratus*, ces granules sont assez grosses ce qu'on peut voir après coloration avec l'hématoxyline de fer, le phloxine ou l'orange G. Les noyaux, relativement volumineux, sont parfaitement sphériques et leurs nucléoles ont une position centrale. Entre ces cordons il y a de nombreux petits vaisseaux et le plus souvant on peut observer une quantité considérable de sinus intercellulaires.

Certainement il s'agit du tissu interrénal de GIACOMINI.

b. Le seconde type de cellule se distingue de différents façons de celui que nous venons de décrire. C'est à dire elles sont organisées en petits

groupes, qui se trouvent entre les cellules interrénales. Après fixation dans le liquide de Bouin ou dans le "Susa" et après coloration par l' hémalun-éosine ou par l'Azan le plasme n'est coloré que très faiblement. Les noyaux d'une forme irrégulière sont pâles et ils ont un aspect enflé. Leurs nucléoles, souvent très petites, dont chaque noyau en renferme une ou deux, sont localisé fréquemment tout près de la membrane nucléaire.

Après fixation dans le Müller-formol, auquel nous avons ajouté de l' acide acétique jusqu'a 5%, et après postchromisation dans du bichromate de potassium à 3%, le cytoplasme renferme un pigment granuleux d'une couleur jaunâtre à brun. Nous sommes d'avis que cette phaeochromie soit la même que GIACOMINI a décrit autrefois. On a vraisemblablement affaire à des cellules chromaffines, l'homologie des cellules médullo-surrénales des Mammifères.

Aucune trace d'une telle pigmentation ne pouvait être aperçu dans le cytoplasme des cellules interrénales. Nous avons pu montrer cette phaeochromie chez *Cottus scorpius*, *Perca fluviatilis*, *Gasterosteus aculeatus* et *Carassius auratus*. La recherche de la présence des cellules phaeochromes est en traîn d'être continué chez d'autres espèces de Téléostéens.

Chez des diverses Mammifères on a étudié l'effet de l'Amphenone non sur l'histologie et sur la physiologie de quelques organes endocrines (HERTZ *et al.*, 1955; HOGNESS *et al.*, 1953: HEMING *et al.*, 1952; TULLNER *et al.*, 1956; ROSENFELD and BASCOM, 1956; MARKS *et al.*, 1958; CHART *et al.*, 1958; HOET *et al.*, 1957). Chez des Oiseaux aussi quelques auteurs ont étudié l'effet de l'Amphenone sur la glande thyroïde (ADAMS and BENNETT, 1953; LEVER and VAN OVERBEEKE, 1956; VLIJM, 1956, 1958).

Quant au cortex surrénal l'Amphenone, administrée soit par voie buccale soit par injection, cause une forte hypertrophie cellulaire accompagnée d'une inhibition importante de la synthèse des hormones corticostéroïdes.

Nous avons exécuté des expérimentations à propos de l'effet de l' Amphenone sur quelques organes endocrines chez des Téléostéens. Nous décrirons ici les résultats concernant le tissu interrénal.

Comme animaux d'expérience sont utilisé: *Betta splendens* Regan, *Gasterosteus aculeatus* L. et *Lebistes reticulatus* Peters. L'Amphenone fut dissolu dans l'eau. Après avoir tué les poissons à la fin de l'expérimentation ils furent fixés in toto. Les pièces, inclusées à la paraffine furent coupées en série à 5 μ d'épaisseur. Les coupes sont colorées par l'hémalun-éosine, par l'hématoxyline de fer, par l'Azan, par l'hématoxyline au chrome-phloxine (Gomori), par le fuchsineparaldéhyde ou par le bleu de toluïdine.

Chez *Betta splendens* (♀♀) l'Amphenone, dans un dosage de 0.0005% après 15 jours a induit une hypertrophie considérable des cellules

iterrénales. En outre une faible hyperplasie se présentait. Un dosage plus fort, c-à-d de 0.005%, dans la même période a causé une hypertrophie plus forte, pendant que l'activité mitotique était augmentée fortement aussi. Afin de préciser quantitativement l'hypertrophie, nous avons fait usage d'une méthode planimétrique. L'activité mitotique a été exprimé comme le pourcentage de mitoses. Pour les résultats de ces mensurations, voir: VAN OVERBEEKE, 1958.

Examination du tissu interrénal chez des epinoches (*Gasterosteus aculeatus* forma *gymnura*, ♂♂) traités dans une même manière avec l' Amphenone (dosage: 0,005–0,01%, durée 15 jours) révélait les mêmes altérations profondes, hypertrophie et hyperplasie des cellules. Chez *Lebistes reticulatus* (♂♂), l'Amphenone administrée également comme chez les deux autres espèces, n'induisait qu'une très faible hypertrophie des cellules interrénales dans une période de 26 jours, pendant qu'après 36 jours cet effet semble avoir disparu. Ici il n'y avait aucune augmentation de l'activité mitotique.

L'étude des cellules phaeochromes de tous ces poissons montrait que chez cettes cellules-ci aucune altération cytologiquement appréciable n'a eu lieu.

Ultérieurement nous publierons des données détaillées sur les résultats chez *Gasterosteus aculeatus* et chez *Lebistes reticulatus*.

L'effet de l'Amphenone sur l'histologie du cortex surrénal chez des Mammifères est attribué à la perturbation de la synthèse des hormones corticales, c-à-d les altérations histologiques seraient des phenomènes secondaires. Chez les Téléostéens le rôle physiologique du tissu interrénal est encore loin d'être connu en détail mais récemment on a décélé la présence des corticostéroïdes dans le sang de la carpe (LELOUP-HATEY, 1958; BONDY, UPTON and PICKFORD, 1957) et dans le sang du saumon (HATEY, 1954; FONTAINE et HATEY, 1954).

Jusqu'ici on n'a pas encore démontré que ce sont les cellules interrénales, qui produisent les stéroides, comme le font les cellules du cortex surrénal chez les Mammifères. Cependant, si nous comparons l'effet de l'Amphenone sur les cellules interrénales à l'effet de cette substance sur le cortex surrénal, il nous semble probable que chez les Téléostéens eux aussi une perturbation de la synthèse des hormones par l'Amphenone soit à la base des altérations histologiquement appréciables.

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2. J. van der Vecht (Leiden). On *Eumenes arcuatus* (Fabr.) and some allied Indo-Australian wasps.

Voir: Zool. Verhand. Leiden, no. 41, 1959.

3. J. de Wilde (Wageningen). Physiological action of the corpora allata of the adult Colorado beetle, *in vivo* and *in vitro*.

In the adult Colorado Beetle, reproduction and diapause are governed by the activity of the corpora allata.

Beetles in diapause show several phenomena characteristic of deficiency of the corpora allata (pseudo-allatectomy). This was tested in experiments with extirpation and reimplantation of the corpora allata in both sexes. These experiments show that the behaviour changes, the arrest of oogenesis and the low rate of respiration characteristic of diapause are all under the direct control of the corpora allata.

This humoral effect is on a subcellular level. Oxygen consumption of homogenates of diapausing beetles is significantly stimulated by addition of 2-4 active corpora allata per beetle equivalent. Small doses of a juvenile hormone concentrate prepared from the abdomens of male *Cecropia* silkworms have the same effect, *in vitro* as well as *in vivo*.

This may be a valuable test for assaying the juvenile hormone.

4. L. M. Schoonhoven (Wageningen). Diapause in *Bupalus piniarius* (Lepidoptera, Geometridae) in relation to host-parasite synchronization.

Although diapause in *Bupalus piniarius* L. (Geometridae) is not obligatory, its break is promoted by chilling. A positive correlation can be established between the duration of chilling and the rate of develop-

ment afterwards. Decerebration of the pupa has no effect on metamorphosis unless accomplished within two days after pupation.

Notwithstanding the non-obligatory character of diapause in *Bupalus*, pupae stored at low temperature maintain a low rate of oxygen consumption, together with a low level of cyctochrome oxidase activity. Both are gradually increased when the pupae are placed at 25° C.

The larva of *Eucarcelia rutilla* Vill. (Tachinidae) hibernates within the host pupa and is apparently activated when adult development of the host is started. Also under experimental conditions the life cycles of host and parasite are synchronised.

It proved possible to activate the larva by chilling early decerebrated, parasitized *Bupalus* pupae. These pupae themselves cannot be activated. This fact suggests that either chilling may directly affect the larva, or that during diapause it is inhibited by the host, the inhibiting factor being removed by chilling.

Séance du 23 mai 1959 à Leiden

1. K. H. Voous (Amsterdam). Les relations entre les avifaunes de l'Europe et de l'Afrique

Voir: Proc. 1st Panafr. Ornithol. Congr. 1957, Ostrich, suppl. 3, 34–39, 1959.

2. W. H. Dudok van Heel (Den Helder). Perception acoustique de la direction chez *Phocaena phocaena*. Voir: Nature, **183**, 1063, 1959.

3. H. Wolda (Groningen). Décroissance de réactions chez *Notonecta glauca*.

Une publication détaillée de cette conférence paraîtra bientôt dans ce journal.

4. C. Naaktgeboren (Amsterdam). Ueber das Zustandekommen der Lage vor der Geburt beim Fötus des Rindes.

Das Hausrind wird in 95 von den 100 Fällen in Kopfendlage geboren. Die Geradlage der Frucht kommt zustande durch die Anbequemungsart des Fruchtkörpers in das Uteruskavum (Akkommodationstheorie). Das Zustandekommen der Kopfendlage ist durch Schwergewichtsverhältnisse im Fötus und durch peristaltische Uteruskontraktionen nicht völlig zu erklären.

Genau wie bei anderen Säugetieren ist das embryonale Wachstum des Rindes nicht proportional. An kleinen Embryonen (etwa 9 Wochen) sind der verhältnismäßig große Kopf, die kleine Steißpartie und die kurzen Extremitäten auffällig. Allmählich entwickelt sich der große Kopf zum relativ kleinen Kopf des neugeborenen Kalbes. Die Steißpartie wird gerade zur größten Körperstelle. Die Extremitäten werden immer relativ länger.

Der Steißumfang ist kleiner als der Kopfumfang in Früchten, jünger als 4 Monate.

Im 5. Monat wird der Steißumfang der größere. Ab 20 Wochen stellen dich die Föten, in Salzwasser schwimmend, mit dem Beckenende am tiefsten. Jüngere Embryonen stellen sich mit dem Kopfende am tiefsten. Im 5. Monat tritt auch die Senkung des Tragsackes ganz deutlich ein und fangen die fötalen Bewegungen an kräftig aufzutreten. Durch diese Bewegungen, durch die Einwirkung der Massenverteilung in der Frucht sowie durch die Raumverhältnisse in der Bauchhöhle, zeigt die Frucht vom Ende des 5. Monat an, eine ständig zunehmende Neigung sich in Kopfendlage zu stellen.

5. L. DE RUITER (Groningen), aussi de la part de C. J. DEN OTTER. Tarsal chemoreceptor activity in *Calliphora erythrocephala* Meig. as an example of nervous messages.

It is generally agreed that conduction, transformation and distribution of messages are the main functions of the nervous system. However, as pointed out, *e.g.*, by McKAY and McCULLOCH, little is known about the exact nature of the nervous message, *i.e.*, on the question which part of the total physical information present in the activity of nerve cells is actually utilized by the receiving stations in the organism. Research is hampered here by the complex nature of nervous information channels, which usually consist of hundreds of pathways woven together into a vast net. The message transmitted is composed of the simultaneous activity in a great many strands of this structure, probably in such a way that it is not merely the presence or absence of activity that counts, but the details of the time pattern of the action potentials in each element (AMASSIAN and WALLER). In this respect, a unique opportunity for investigation is offered by the tarsal and labellar chemoreceptors of flies, which transmit messages capable of triggering complete behaviour patterns over a very few, or even over a single nerve fibre.

With this in mind, we are measuring, on the one hand, the input into the central nervous system from these receptors, and, on the other hand, certain activities of the fly released by this input. A study of the correlations between sensory input and time pattern of activities may be expected to shed some light on the question raised above.

Only some results of the measurements of receptor activity will be reported here, activity being expressed in action potential frequencies in the individual sensory nerve fibres. The experiments were made partly on amputated legs, partly on intact immobilized flies. As shown by HODGSON and ROEDER, DETHIER, and others, of the two types of chemoreceptor cells located in tarsal hairs of flies, one (the + cell) transmits messages releasing sucking behaviour, and the other (the — cell) transmits messages giving rise to avoidance in the fly.

Therefore, the external stimulus situation for the fly in this respect

can best be stated in terms of the extent to which the environment is fit to be sucked, and the extent to which it should be avoided. Neither of these two measures of the external situation is a simple function of the concentration of individual chemical substances in the environment. In addition to the concentration of the substance tested, at any given moment at least four factors influence the intensity of the response of a given receptor, which response must somehow contain the meaningful message for the central mechanisms:

a. The + cell and the — cell respond each to a variety of substances, of which we have tested only sucrose (which primarily affects the + cell) and NaCl (which mainly stimulates the — cell). Now both in the work of others and in our own data, there is evidence that the response to one substance of a given cell may depend in part on the concentrations even of substances that do not themselves provide adequate stimulation for that cell.

b. Depending on concentration, a single substance may activate either the + cell, or the — cell, or both. In our experiments, we found a positive correlation between + cell activity and stimulus concentration in the case of sucrose, but in the case of salt there appears to be a negative correlation.

c. The response of the chemoreceptors is probably also influenced by the internal state of the fly. In the responses of the — and + cells to salt, we found variations that can hardly be accounted for in any other way.

d. Obviously, the signals transmitted by a receptor at a given moment also depend on its degree of adaptation. In our experiments, + cells in about 1 ½ min. reach a constant response level, which is maintained for periods of 10 min. and more, and which is correlated with stimulus concentration. The — cells on the other hand, adapt within one minute to a very low level of activity, which is no longer clearly correlated with stimulus concentration, even though initial activity is strongly concentration dependent. This difference may well be related to the functions of the messages transmitted: under normal conditions prolonged — cell activity will not be required, as avoidance will remove the receptor from the source of stimulation.

Séance du 29 juin 1959 à Leiden

G. Thorson (Helsingør). The balance between invertebrate predators and their prey animals on the sea bottom.

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 2 octobre 1959 à Dordrecht

1. I. S. Zonneveld (Sleeuwijk). Le biotope du Biesbosch et son histoire. *Voir:* Akademiedagen Kon. Ned. Akad. Wet. X, 93–113, 1957.

2. K. F. VAAS (Yerseke). Hydrobiological observations in the Biesbosch.

Preliminary hydrographical conclusions, derived from data obtained during a brief study carried out by a team of hydrobiologists (representatives of various hydrobiological and academic Institutes and students) in the fresh-water tidal swamps known as the Biesbosch and situated in the estuaries of the Rhine and the Meuse, can be summarized as follows.

As compared with the Meuse, the Rhine is characterized by water of reduced oxygen contents, enlarged BOD-5 values and mineral contents, owing to pollution by the German industrial areas.

The small part of the Biesbosch, west of the Nieuwe Merwede, known as the Zuidhollandsche Biesbosch, is not transversed by running water, but a mixture of Rhine-water from the Nieuwe Merwede and water from the Hollandsch Diep is pushed in and out by the action of the tides.

In the Hollandsch Diep a wedge of heavy, brackish water extends from Zype to the railway bridge of Moerdijk, at high tide, and does not reach the Biesbosch itself. Tidal influence, as witnessed by oscillations of the waterlevel, is felt beyond the entire area. As a result of strong winds from the South-West, water from the Meuse is sometimes pushed backwards into the Nieuwe Merwede, which river then shows a lower salinity near its connection with the Amer than further downstream.

The main part of the area east of the Nieuwe Merwede, and known as the Brabantsche Biesbosch, has a water-regime mainly governed by the Meuse and only slightly influenced by the Rhine. In the extreme eastern regions (Bakkerskil) a low chlorinity of the water indicates almost pure influence of the Meuse. If there is a part governed by the Rhine only, that area must be situated N-E of Kop van 't Land, where no samples were taken. Compared with the riverwater surrounding the area, the interior of the Biesbosch shows an improved oxygenation — often rising to supersaturation — and markedly reduced amounts of phenol are found in the water. Both findings are the result of the purifying action of the slower running water with more vegetation.

As chlorinity in the inner parts of the Biesbosch exceeds the 100 mg/l level in most places, it is questionable whether the Biesbosch may be regarded as a true freshwater area.

Pending further analysis of the plankton-data, to be carried out by members of the Hydrobiological Society, the following remarks can be made concerning the Rotatoria and the littoral macrofauna.

In both rivers surrounding the Biesbosch, as well as in the Hollandsch Diep, *Brachionus calicyflorus* is the principal rotifer and also in the Zuidhollandsche Biesbosch, but in the Brabantsche Biesbosch *Brachionus angularis* comes to the fore.

In the Oostkil and the Bakkerskil the main rotifer was a *Trichocerca* species, also found in the Amer near the mouth of the creek, but not further downstream. *Polyarthra remata*, found together with *Trichocerca*, spreads from its origin in the eastern smaller creeks, throughout the Amer untill the Hollandsch Diep.

In a small pool near Willem III, not connected with the main creeks and showing a lower salinity than its surroundings, *Conochilus unicornis* was observed, together with *Pompholix* and various *Cladocera*, organisms common in dead river-arms of the Meuse according to REDEKE.

On the naked mud-flats in the S-W part of the area, a film of *Euglena* and blue-green algae with *Navicula pygmea* was observed.

The latter diatom is a slightly brackish species.

In the supralittoral zone *Orchestia cavimana* and the Mollusc *Succinea* was common.

In the tidal zone of the Biesbosch the small Prosobranchiate *Pseudamnicola confusa* was found, the first time this animal was encountered alive in the Netherlands. The leeches *Herpobdella octulata* and *Trocheta bykowskii* also live in this zone.

A frequent sublittoral organism is *Cordylophora caspia*, but in the more brackish water west of the area this organism is better developed.

The brackish species *Gammarus zaddachi* is found far into the Biesbosch owing to lack of competition with *Gammarus pulex*, the fresh-water form, only found in isolated pools in the interior of the Biesbosch, as this organism seems to avoid running water. Frequently encountered were *Gobius microps* and young flounders.

3. P. C. HEYLIGERS (Utrecht). Soil fauna of the osier beds of the Brabantse Biesbosch[1].

ZONNEVELD has demonstrated that the tidal movements of the fresh water are one of the most important ecological factors in the Biesbosch, affecting both vegetation and soil. The main purpose of the present investigation was to examine the influence of this factor on the structure of the soil fauna.

The soil fauna was sampled in two ways: by sieving soil samples under water (all animals bigger than 1.5 mm being retained), and by trapping beetles and other "macro-arthropods", moving on the surface, in tins dug into the ground.

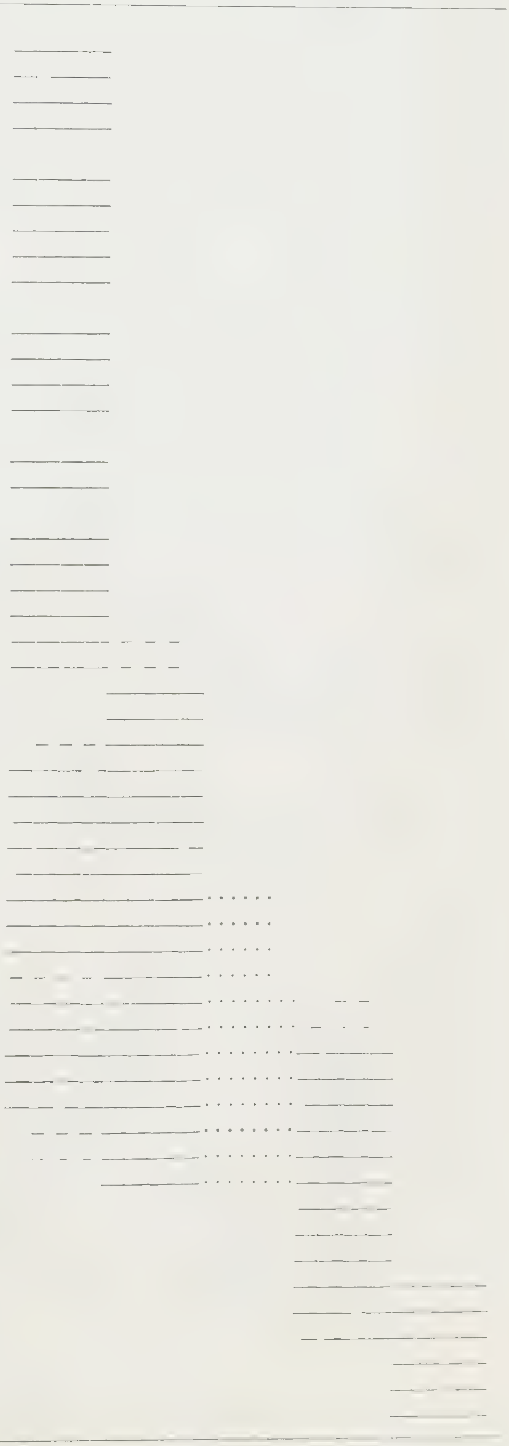
In the table, the distribution of the most important species, *c.q.* other taxa, in the osier bed biotope is summarized. In the lowest localities, *i.e.*, those most often inundated at flood tide, a population occurs which is normally found on constantly submersed places. Acord-

[1] This paper contains the results of a preliminary investigation of limited scope, carried out in 1953.

Soil fauna types of the osierbeds of the Brabantse Biesbosch

	Myriapoda-type	*Eiseniella*-type	intermediary type	*Pisidium*-type: *Hydrobia*-subtype	*Pisidium*-type: *Tubifex*-subtype
Flooding frequency in %	8 — 30	30 — 60	60 — 85	85 —	— 100
Height in cm with regard to Mean High Tide	+ 35 — + 10	+ 10 — — 10	— 10 — — 40	— 40 —	— — 70
Average watercontents of top-soil (g water/100 g oven-dry soil)	50	75	?	120	150–300

Lumbricus rubellus (Oligochaeta-Lumbricid.)
Dendrobaena sp. (do.)
Allolobophora spp. (do.)
Haplophthalmus mengei (Isopoda)
Necrophloeophagus longicornis (Chilopoda)
Brachydesmus superus (Diplopoda)
Brachyiulus littoralis (do.)
Symphylella vulgaris (Symphyla)
Forficula auricularia (Dermaptera)
Carabus granulatus (Coleoptera-Carabidae)
Platysma nigrum (do.)
Platysma vulgare (do.)
Elateridae larvae (Coleoptera)
Itonididae larvae (Diptera-Nematocera)
Tipulidae larvae (do.)
Empididae larvae (Diptera-Brachyc.)
Retinella nitidula (Gastropoda)
Patula rotundata (do.)
Cochlicopa lubrica (do.)
Tricladida terricola (Platyhelm.)
Trichoniscoides albidus (Isopoda)
Eiseniella tetraedra (Lumbricidae)
Vallonia spp. (Gastropoda)
Orchestia cavimana (Amphipoda)
Haemopis sanguisuga (Hirudinea)
Metatrichonisc. leydigii (Isopoda)
Staphylinidae larvae (Coleoptera)
Cantharidae larvae (do.)
Itonididae larvae, other sp.
Hygromia hispida (Gastropoda)
Vitrea crystallina (do.)
Succinea spp. (do.)
Carychium minimum (do.)
Agonum assimile (Carabidae)
Anchus ruficornis (do.)
Mermis sp. (Nematoda)
Enchytraeidae (Oligochaeta)
Dryopidae larvae (Coleoptera)
Trocheta bykowskii (Hirudinea)
Candona sarsi (Ostracoda)
Limnaea truncatula (Gastropoda)
Hydrobia jenkinsi (do.)
Pisidium obtusale (Lamellibranch.)
Sphaerium corneum (do.)
Pisidium nitidum (do.)
Pisidium cinereum (do.)
Pisidium personatum (do.)
Tubificidae (Oligochaeta)
Pisidium subtruncatum (Lamellibr.)
Pisidium milium (do.)



ing as sites are higher situated, and consequently less frequently flooded, the character of the community changes into that of a land fauna. This differentiation makes it possible to distinguish four soil fauna types.

The "Myriopoda-type" is characterized by a rich population of Lumbricidae (275–100 specimens/sq.m.), by the occurrence of Chilopods, Diplopods and bigger Carabid beetles, and by the fact that the larvae of several insect families occur in this type only. The soil inhabited by this community is a well aerated and ripened clay soil, with a crumbly structure in the upper layers.

The lower situated "*Eiseniella*-type" is characterized by the occurrence of *Eiseniella* (20–90 spec./sq.m.), *Vallonia* and *Orchestia*, and a large number of *Carychium* (2400–3700 sec./sq.m.). There are several species in common with the "Myriopoda-type". The clay soil is less ripened and aerated and has on the average a higher percentage of water.

The low situated "*Pisidium*-type" is characterized by various *Pisidium* species (75–1800 spec./sq.m.) and is divided into two subtypes: the "*Hydrobia*-subtype" with *Hydrobia jenkinsi* as characteristic species (100–2200 spec./sq.m.) and with several species in common with the less frequently flooded types, occurring on rather soft, badly aerated clay soil, and the "*Tubifex*-subtype" in which the Tubificidae play a prominent part (10,000–40,000 spec./sq.m.), and which is confined to the very soft completely reduced soils.

Between the "*Eiseniella*-type" and the "*Pisidium*-type" we expect an intermediary type, but we do not have at our disposal representative samples from places with a flooding frequency between 60% and 85%.

Investigating the vertical distribution in aerated soil, the highest level of the groundwater appeared to coincide broadly with the lower limit of the distribution. Only a small number both of specimens and of species occurs down to this depth and most animals live in the uppermost soil layer.

Remarks about some species:

The leeches *Haemopis sanguisuga* and *Trocheta bykowskii* are predators of worms (Lumbricidae, Enchytraeidae, Tubificidae). In Holland *Trocheta* is known from a few localities only. On the suitable places in the osierbeds their number is 50–150 spec./sq.m.

The Biesbosch is the only find-spot in Holland of the Ostracod *Candona sarsi*. For the rest this species is known from five localities in Germany and one in Norway. In the "*Eiseniella*-type" 60 spec./sq.m. occur, in the "*Hydrobia*-subtype" 450 spec./sq.m.

Among the Isopods the specimens of *Trichoniscoides albidus* are some of the first ones sampled in the Netherlands. During 72 years the

species *Metatrichoniscoides leydigii* was missing because as well the type-specimens as the type-locality were lost. Meanwhile this species was described under a new name which is withdrawn after the rediscovery. At present *Metatrichoniscoides* is known from four find-spots, located in Sweden, Finland, Holland and France. In the Biesbosch a few hundred spec./sq. m. occur in the "Myriapoda-type" and the "*Eiseniella*-type".

It thus appeared from my investigation not only that for the soil fauna, too, the tidal movement is a very important factor, but also that the Biesbosch is a favourable biotope for several rare and, from an ecological point of view, almost uninvestigated species.

For further details, see:

HEYLIGERS, P. C., "Biosociologische studies in de Brabantse Biesbosch", 1955 (stencil R.I.V.O.N.), and

HEYLIGERS, P. C., T. LEBRET, C. J. VERHEY and I. S. ZONNEVELD, "De Biesbosch, land van het levende water", in the press (Thieme, Zutfen).

4. T. LEBRET (Middelburg). Les oiseaux du Biesbosch.

Un résumé de cette conférence n'a pas été remis au secrétaire.

5. C. J. VERHEY (Dordrecht). La pêche dans le Biesbosch.

Un résumé de cette conférence n'a pas été remis au sécretaire.

6. A. VAN WIJNGAARDEN (Bilthoven). Les mammifères du Biesbosch.

Un résumé de cette conférence n'a pas été remis au secrétaire.

Séance du 31 octobre 1959 à Utrecht

1. J. J. WILLEMSE (Amsterdam). The way in which flexures of the body are caused by muscular contractions, especially in fishes.

The flexures of the body are caused by contractions of a muscle-system consisting of longitudinal muscle fibres interrupted by sheets of connective tissue, the myosepta. Authors dealing with this subject accept the existence of a kind of "muscle-tendon-system": The forces brought about by muscular contraction are transmitted to the vertebrae by the myosepta. One of the points not in accordance with the "muscle-tendon" theories is the fact that as a rule the myosepta are very thin near their point of attachment to the vertebrae.

It is possible to give an explanation for the origin of flexures of the body or parts of the body by comparing the distribution of forces in the animal structures with the distribution of forces in a bimetal. Both structures show a similar distribution of longitudinally directed internal forces. The presence of these forces results in a bending of the body. In this explanation no attachment of the myosepta to the vertebrae is required.

A full account has been published in: Proc. Kon. Ned. Akad. Wet. **C 62,** no., 5 1959.

2. Mme. C. VERHEYEN-VOOGD (Utrecht). L'influence de la reine

sur le comportement et sur le développement des ovaires chez les abeilles ouvrières.
Voir: Thèse Utrecht, 1960.

3. R. Boddeke (Amsterdam). Histological characteristics of the body-musculature of fishes in connection with their mode of life.

In fishes there are two types of muscle fibres, *viz.:*
a. broad, white fibres without myohemoglobin and fat, with low sarcomeres and a poor vascularization;
b. narrow fibres with fat, with high sarcomeres and a great amount of capillaries. These fibres are red if they occur in a well-developed lateral line-strip. They are white if this strip is poorly developed.

In principle fishes are sprinters but, in connection with their mode of life, some species have more or less developed staying powers. In all fishes by far the majority of the body-musculature consists of broad white fibres. The more qualities of the stayer a species shows, the better the narrow red fibres are developed. The broad fibres principally possess the qualities required for the sprinter, the narrow red fibres those required for the stayer. The height of the sarcomeres is probably correlated with the speed of contraction. These researches are published in Proceedings Kon. Ned. Akad. Wetensch. Amsterdam, Ser. C, **62,** No. 5, 1959.

4. D. Hillenius (Amsterdam). Ciliary reversal in *Metridium* under nervous control.

Parker (1905, 1928) found that the cilia on the oral disc of *Metridium dianthus* (L.) are able to perform a reversal of the common movement under the stimulating influence of certain materials. Ordinarily the cilia beat outwards from the mouth towards the periphery, but broth of mussels or fish, pepton, deutero-albumose, kreatin, glycogen and KCl cause a reversal, so that the tested material is moved from the tentacles to the mouth and eventually is swallowed. With Mg SO_4 Parker eliminated the nervous control and he found that the ciliary reactions remained the same, *viz.* outflow of neutral carmine powder and inflow of broth coloured with carmine.

However, several observations are hardly explicable without assuming some nervous influence. For instance, hungry anemones may change their habit by ingesting materials which are ordinarily refused. "Hungry anemones may swallow even stones" (Prosser). It might be imagined that all the cells of the oral disc together feel the same degree of hunger and therefore change their reaction in the same way and at the same time. But sometimes one finds anemones "hesitating" between the common reaction and the hunger reaction:

To six specimens I administered some broth with quinine (coloured with carmine), which is ordinarily refused. Two of them refused in-

deed, two brought it to the mouth, where it disappeared in the oesophagus. Of the remaining two one firstly refused *i.e.* brought the material to the periphery, then there originated an inflow to the mouth, again reversal and so on. After five negative reactions, alternating with four positive reactions, the material was discharged definitely. The last anemone did the reverse: it performed four positive reactions, alternating with three negative ones, and swallowed the broth and quinine with the last movement.

This means that the reaction of so many ciliary cells is remarkably equal. It would be possible to assume that the cells are hesitating individually, but the fact that so many cells together give one moment a positive reaction and another moment a negative one, can only be explained by coordination which in Coelenterates is only conceivable by means of the nervous system. I tried to repeat PARKER's experiments with drugged anemones. PARKER did not mention how he knew when the nervous control was sufficiently eliminated. At first I thought that this could be concluded from the reactions of the anemone, for instance after pincing tentacles or the oral disc. In normal condition the animal is easily irritated, reacting with contraction of tentacles or even of the whole body. So when an anemone is drugged with $MgSO_4$ and no reaction to pincing is visible, one could suppose that the nervous control is eliminated.

With normal (not hungry) anemones I could confirm PARKER's observations: no influence of the drug on the ciliary reversal.

Four hungry anemones (reacting positively to neutral carmine powder) were drugged with $MgSO_4$ until the tentacles did not react to mechanic stimuli. In this condition the neutral carmine was refused, broth accepted. After 18 hours the anemones regained their normal condition (reacting to mechanic stimuli) and again accepted neutral carmine.

Three times these four anemones were drugged. Each time they lost their "hunger behaviour": elimination of nervous control causes the disappearance of the adaptation to hunger. This means that the effective stroke of the cilia is influenced at least partly by the nervous system.

Several observations convinced me that the animals were not yet sufficiently drugged. In all cases mentioned above I found that the tentacles which did not react to mechanical stimuli, remained sensitive to chemical stimuli. The tentacles of drugged animals reacted positively (though slower than normally) to broth: they bend to the mouth. Therefore I tried to administer a higher concentration of $MgSO_4$, in order to annihilate the reaction to chemic stimuli too. This could be achieved. Three anemones became very slack. One specimen

drooped its oral disc. In this condition both neutral carmine and broth were refused. Three hungry anemones in completely drugged condition refused neutral carmine and bouillon.

Conclusion: Though the movement of the cilia on the oral disc of *Metridium dianthus* is autonomous, the adaptation to the hungry condition and the reversal of the effective stroke is caused by nervous agency.

References:

Parker, G. H., 1905: The reversal of ciliary movement in Metazoans. Amer. Journ. Physiol., Boston, Mass, **13,** 1–16.

Parker, G. H. and A. P. Marks, 1928: Ciliary reversal in the sea-anemone *Metridium.* Journ. Exp. Zool., Philadelphia, **52,** 1–6.

5. W. M. Herrebout (Wageningen). Host selection in the parasitic fly, *Eucarcelia rutilla* Vill.

Two aspects of adaptation in the searching behaviour of the gravid female for hosts (larvae of pine looper moths, mainly *Bupalus piniarius* L.) are discussed.

In the oviposition period the larvae of *Bupalus* have reached their 2nd or 3rd instar. Direct observation showed that during day 85% and 50%, respectively, of these instars are resting at the top of the needles of the Scots Pine. Laboratory experiments revealed that hosts living at the top of the needles run a greater risk of being parasitized than larvae living deeper among them. It also seems that parasites searching for hosts do not waste time by looking for them in places where they are normally absent. This view is supported by field data on parasitization in *Semiothisa*, *Ellopia* and *Thera obeliscata*. In the laboratory these species were as attractive as *Bupalus*, but in the field in 1958 only *Semiothisa* reached the same level of parasitization as *Bupalus*. Especially *Thera* showed a very low degree of attack. Observations in 1959 showed that larvae of *Ellopia* and *Thera* live deeper among the needles. Though both species are unsuitable as hosts, it seems that the fly escapes ovipositing on them only because of their more hidden resting place.

In placing the eggs upon the host's body, the female shows a very clear preference for the first three segments. This has a double advantage: the "eating off" reaction (to which about 20% of the eggs laid more caudally fall victim) is prevented, and the chance that the development of the parasite larva will be impossible because of internal resistance of the host is lowered by about 25%.

Séance du 20 et 21 novembre 1959 à Amsterdam, organisée en collaboration avec la Société Royale Néerlandaise de Botanique et

la Société Génétique Néerlandaise, sous les auspices du Conseil Biologique Néerlandais.

1. A. G. van Melsen (Nijmegen). Introduction générale.
2. M. J. Sirks (Groningen). Les bases de la variabilité.
3. L. de Ruiter (Groningen). L'évolution surprise: Mesurage de la sélection dans la nature.
4. J. C. Dorst (Wageningen). L'amélioration des plantes cultivées et l'évolution.
5. I. M. van der Vlerk (Leiden). La paléontologie et l'évolution.
6. J. van der Vecht (Leiden). La systématique et l'évolution.
7. G. H. R. von Koenigswald (Utrecht). L'évolution de l'homme.
8. A. Quispel (Leiden). L'origine de la vie: reconnaissances dans l'inconnu.

L'ensemble de cettes conférences sera publié dans un livre qui apparaîtra dans le printemps de 1960.

Séance du 19 décembre 1959 à Amsterdam

1. J. W. Huismans (Utrecht), aussi de la part de J. H. Veerkamp. A tracer study on the metabolism of lipids and amino acids in the green frog (*Rana esculenta* L.) and the carp (*Cyprinus carpio* L.) with Na-acetate-1-C^{14}.

In order to investigate whether the metabolic pathways are indeed the same in all organisms as is sometimes supposed, two representatives of the lower Vertebrates, but scarcely examined in this respect, were chosen. Like Schoenheimer (1942) and many others, we chose the tracer method. Because of the central position of acetate in metabolism we worked with injections of labelled acetate.

Five frogs and 1 carp were injected once a day during a period of respectively 6 and 7 days with an almost physiological solution of sodiumacetate-1-C^{14} until a total of 150 μC for the frogs and the same amount for the carp alone was reached. After this period the animals were killed.

A mixture of saturated and unsaturated fatty acids, and cholesterol were separately isolated from some groups of organs. Furthermore a number of amino acids were isolated from the frogs' residue.

The fatty acids derived from the liver of both kinds of animals proved to be much more radioactive than those derived from the muscles and other organs. Thus we may consider the liver as the chief-organ for the synthesis of amino acids.

In both cases the saturated fatty acids were higher labelled than the mixture of the saturated and unsaturated fatty acids; the carboxyl-group of the saturated fatty acids was very highly labelled in comparison to the total of the carbon atoms. Probably the animals elongated

only the fatty acid chains in consequence of the low rate of metabolism of these cold-blooded animals (the experiments were carried out in winter). However, the fact that the radioactivity of the expired CO_2 (obtained once during the injection period) was high, points to a great activity of the citric acid cycle. Consequently the acetate degraded in this cycle could not be used for synthesis of higher fatty acids.

As cholesterol appeared to possess a low, but significant radioactivity for both kinds of animals, it is proved that these animals are able to synthesize cholesterol starting from a 2 C-compound, in contradistinction to the larvae of certain insects (Sedee 1956).

In the case of the frog the following amino acids appeared to be radioactive: glutamic acid, aspartic acid, alanine, serine, proline, glycine and arginine. No radioactivity was found in phenylalanine, tyrosine, leucine, isoleucine, lysine, histidine, valine and hydroxyproline.

This means that the amino acids which in other animals have found to be essential are not labelled, whereas the non-essential amino acids have taken up the tracer (with the exception of tyrosine and hydroxyproline). As in higher Vertebrates tyrosine is derived from the essential phenylalanine, this way of formation may explain that tyrosine is not labelled. The lack of tracer in hydroxyproline (non-essential in other animals) can be explained by the fact that hydroxyproline occurs almost exclusively in the protein of connective tissue which has a very slow metabolism.

References:

Schoenheimer, R., 1942: The Dynamic State of Body Constituents.

Sedee, Ph. D. J. W., 1956: Dietetic requirements and intermediate protein metabolism of the larva of *Calliphora erythrocephala* (Meig.), diss., Utrecht.

2. Mlle. B. Baggerman (Groningen). Étude comparée des causes de la migration chez l'épinoche et le saumon.

Voir: Arch. néerl. Zool., **12**, 105–318, 1957; Symp. Comp. Endocrinol. (A. Gorbman, editor), New York, 1959; J. Fish. Res. Board Canada, 1960 (in the press); Symp. zool. soc. London I, 1960 (in the press).

3. J. H. Stock (Amsterdam). La formation des espèces chez les parasites.

Un résumé de cette conférence n'a pas été remis au secrétaire.

4. W. Scharloo (Leiden). Sélection expérimentale chez *Drosophila melanogaster* Meig.

Une publication détaillée de cette conférence apparaîtra ultérieurement dans ce journal.

5. F. J. Oppenoorth (Utrecht). Modification à base génétique d'un enzyme causant la résistance contre des insecticides.

Voir: Oppenoorth, Entomologia exp. et appl., **2**, 1959; Van Asperen and Oppenoorth, Entomologia exp. et appl., **3**, 1960.